Genetic variability of drought tolerance of trees of agronomic interest : the role of vulnerability to xylem cavitation

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Variabilité génétique de la tolérance à la sécheresse d'arbres d'intérêts agronomiques: rôle de la vulnérabilité à la cavitation du xylème.

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Résumé

Dans un contexte de changements climatiques, le stress hydrique et la gestion de l'eau sont considérées comme une contrainte importante pour le secteur agricole. Ainsi la sélection pour la tolérance à la sécheresse est devenue un objectif majeur pour de nombreux programmes de sélection. La vulnérabilité à la cavitation est considérée comme un trait d'intérêt pour la sélection à une sécheresse extrême, en particulier pour les plantes ligneuses. Pourtant, l'étendue de sa variabilité et sa relation avec la tolérance à la sécheresse sont mal documentées à l'échelle intra-spécifique, et en particulier dans les espèces cultivées. Dans cette étude, la variabilité génétique de la vulnérabilité à la cavitation a été étudiée sur trois dispositifs expérimentaux différents avec trois arbres d'intérêt agronomique qui sont menacés par le risque de sécheresse: noyer, hévéa et pommier. Une faible ou aucune, variation de la vulnérabilité à la cavitation a été trouvée dans les espèces étudiées ainsi qu'entre deux espèces de noyers malgré les différences de traits précédemment rapportés. Ces résultats suggèrent une canalisation de la résistance à la cavitation dans les organes critiques (branches, tiges). Chez le pommier, le porte-greffe est soupçonné de provoquer de la plus étroite résistance à la cavitation sur le greffon. Par contre, des différences ont bien étés trouvées sur d'autres traits de réponse à la sécheresse comme la régulation stomatique, la chute des feuilles ou encore la vulnérabilité à la cavitation dans le pétiole. Ainsi, la vulnérabilité à la cavitation des organes critiques n'est pas un paramètre pertinent pour la sélection de la tolérance à la sécheresse, et les programmes de sélection sur les espèces étudiées ne semblent pas avoir affecté ce paramètre.

Mots-clés: conductance stomatique, *Hevea brasiliensis*, hydraulique, *Juglans* spp, *Malus domestica*, stress hydrique, sécheresse, vulnérabilité à la cavitation.

Abstract

In a context of climatic changes, drought stress and water management are regarded as one of the most important constraints for agricultural sector. Thus the selection for drought tolerance became a main objective for many breeding programs. Vulnerability to cavitation is considered a trait of interest for the selection for extreme drought stress, especially for woody species. However, the extent of its variability and its relation to drought tolerance are poorly documented on intraspecific level, particularly for cultivated species. In this study the genetic variability of vulnerability to cavitation was studied on three different experimental devices with three trees of agronomic interest that are threatened by the risk of drought: walnut, rubber and apple trees. Low or no variation in xylem vulnerability to cavitation was found in the studied species and between two species of walnuts despite differences previously reported features. These results suggested a canalization of cavitation resistance on critical organs (branches and stems). In apple tree, the rootstock was suspected to cause the narrow resistance to cavitation on the scion. On the contrary, differences on other traits in response to drought stress such as stomatal regulation, leaf shedding or vulnerability to cavitation on petiole were found. Therefore, vulnerability to cavitation of critical organs was not a relevant parameter for the selection of drought tolerance and breeding programs on the species studied did not appear to affect this parameter.

Keywords: drought, *Hevea brasiliensis*, hydraulic, *Juglans* spp., *Malus domestica*, stomatal conductance, vulnerability to cavitation, water stress.

List of abbreviations

A_{sw}	sap wood sectional area
A_v	lumen vessel area
b	maximum conduit's span
D	diameter
DRI	drought risk index
F	flow
$FTSW_c$	critical fraction of transpirable soil water
g_s	stomatal conductance
$g_{s\,12}$, $g_{s\,50}$, $g_{s\,88}$	pressure causing 12, 50 and 88% of stomatal closure
h^2_b	broad sense heritability
K	hydraulic conductance
K_h	hydraulic conductivity
K_s	specific hydraulic conductivity
LA	leaf area
LMA	leaf mass per area
LS	relative shed leaf area
MAP	mean annual precipitation
P	pressure
P_{12},P_{50}, P_{88}	pressure causing 12, 50 and 88% loss of xylem conductivity
PAR	photosynthetically active radiation
PLC	percentage loss of xylem conductivity
R	resistance
r	radius of curve meniscus
RH	relative humidity
ROS	reactive oxygen species
$RRIT$	Rubber Research Institute of Thailand
s	slope of regression line
SEM	scanning electron microscope
$SPAC$	soil-plant-atmospheric continuum
SWC	soil water content
T	absolute temperature, temperature
t	thickness of the vessel separating wall
VC	vulnerability curve
VD	vessel density
V_m	partial molar volume of water
VPD	vapor pressure deficit
α	contact angle between water meniscus and the pore
WD	wood infradensity
γ	water-air surface tension

η	viscosity of water
τ	surface tension of water
Ψ	water potential
$\frac{e}{e_0}$	air relative humidity
ρ	water density
ω	angular velocity

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GENERAL INTRODUCTION

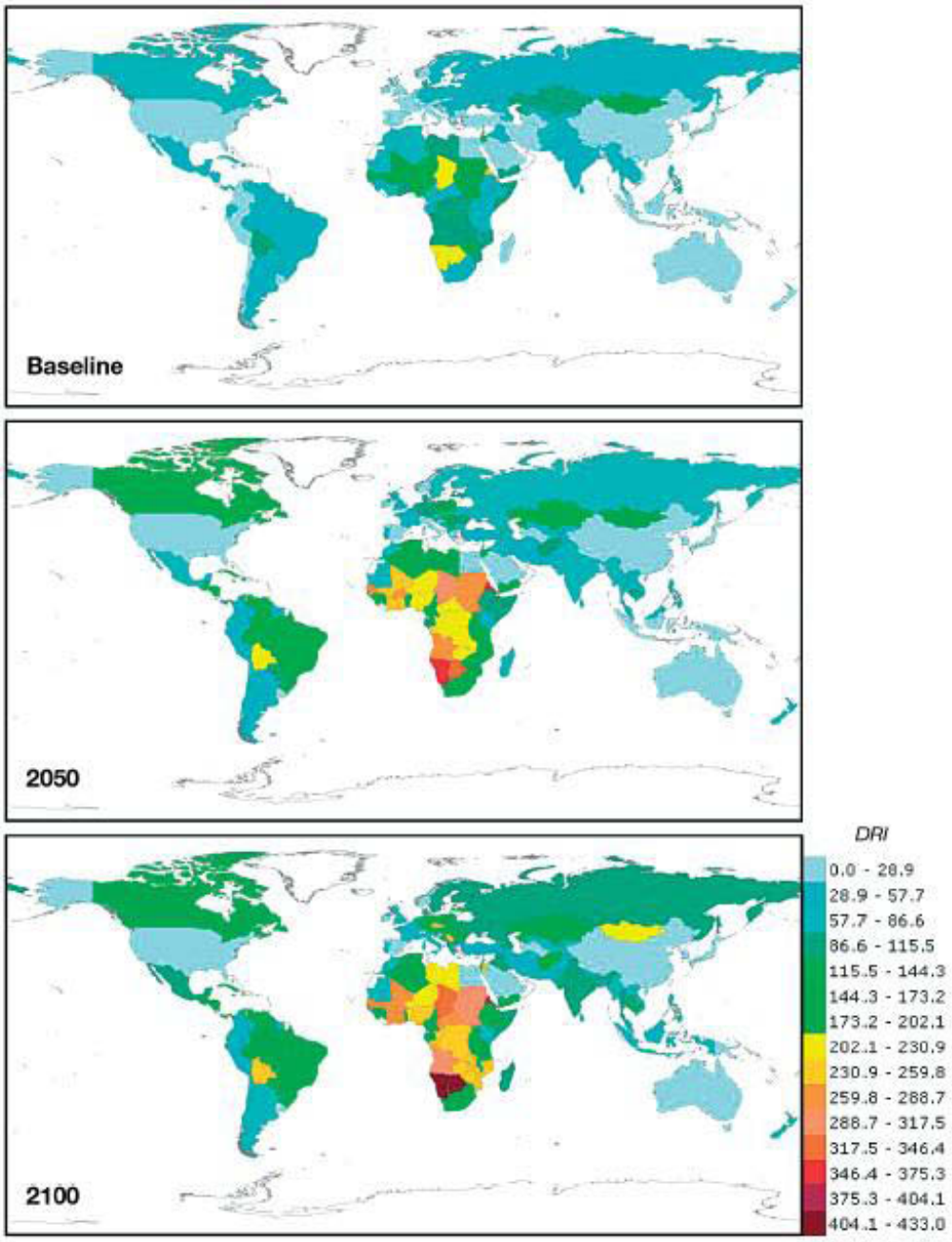

Figure 1. Global drought risk index (*DRI*) of cropland.

The country-specific global drought risk index (*DRI*) is shown for baseline (1961−2006) and projections for 2050 and 2100. It is calculated using the probability of drought disaster, drought degree, and cropland management and irrigation levels of the given place. From Li *et al.* (2009).

Risk of drought stress is predicted to increase in the coming future by several climate models (IPCC, 2013) as a result of the increasing surface temperature and the decreasing precipitation. However, the degree of drought intensity and the duration are expected to differ between regions depend on the specific changes in meteorological factors for the given area. Figure 1 shows the projections of global drought risk index (*DRI*), it is considered as one of the parameters indicating effect of meteorological drought on yield reduction. To obtain this value, the computation taking into consideration the probability of drought disaster, drought degree, and cropland management and irrigation levels of the given place (Li *et al.*, 2009). According to the study, projections indicate globally rising *DRI* for 2050 and 2100: Africa is categorized as a continent with the highest *DRI* value while Oceania is ranked as the lowest. Europe will experience the largest change with 157.53% and 267.84% increasing in *DRI* for 2050 and 2100 respectively while East Asia will have the smallest change in *DRI*: 24.90% for 2050 and 48.28% for 2100. Drought stress hence regards as one of important constraint in agricultural sector.

In order to keep up with the increasing demands of agricultural products, the expansions of agricultural activities into marginal areas have been observed (Glantz, 1994). The extensions could be explained by limited lands for the cultivation in suitable areas, labors availability, or competitiveness of the crop compared to other activities. Developing agricultural activities in marginal areas might contribute to a distribution of jobs leading to better livelihood of local people and prevent labor migration into cities. However, it put natural resources, especially water, under constraints since cultivated species might require more water to obtain the optimal yields. As a result, the ability to withstand drought stress while optimizing water and nutrition uses is of great interest for breeding program.

Water plays the important role to maintain turgor pressure and functions of chemical and biochemical processes in plants. Under stress of water deficit, these processes are affected. Growth is one of the first processes disrupted by drought stress, since cell division, cell elongation and expansion required the turgor pressure in the cells (Nonami, 1998). Yield loss is another important drought effect on plant. Drought-induced stomatal closure is attributed in this reduction in yield because it limits CO_2 assimilation during photosynthesis (Chaves, 1991). In case of severe drought stress, it could induce plant mortality (Allen *et al.*, 2010). Although such level of stress is less investigated, it is of great importance for long-living species including trees.

Species respond differently to drought stress; some could withstand higher level of water deficit and/ or survive longer period of drought stress than others via different mechanisms (Klos *et al*., 2009; McDowell *et al*., 2008; Mueller *et al*., 2005). Therefore the resistance to drought stress appears to be a factor designing not only species habitat but also their performance. It is considered as a pertinent factor for species survival especially for long-living tree species since they might be exposed to several drought stress events in their lifetime.

Traditional breeding and selection programs for agronomic tree species are time consuming tasks due to long-immature phase of the species. As a consequence, the potential traits for drought tolerance screening in tree species have to take less time to investigate, easier to assess and relate with yielding potential. Despite the existence of many traits related to drought resistance that could be used for the selection, not many of these traits were tested especially in agronomic species. The suitable traits should have following characters: (*i*) easy to assess on large-scale, (*ii*) to allow the identification of the genotypic variation, (*iii*) to have a sufficient heritability, and (*iv*) to allow breeders to identify the best and worst genotypes (Pita *et al.*, 2005). Among the potential traits, xylem vulnerability to cavitation is considered a pertinent criterion for drought tolerance screening (Choat *et al.*, 2012; Maherali and DeLucia, 2000; Matzner *et al.*, 2001). With the recent improvement on analytical techniques: Cavitron (Cochard, 2002b; Cochard *et al*., 2005) and air-injection (Ennajeh *et al*., 2011b), xylem vulnerability to cavitation could be rapidly estimated without submitting plant material to drought stress. These technical advances enhance the potential of using vulnerability to cavitation as a trait for drought tolerance screening.

Water in xylem conduits is transported under tension. In this metastable state, it is susceptible to cavitation (Tyree and Sperry, 1989b). The increasing xylem tension due to drought stress could cause the cavitation which is an expansion of air bubble that could fill the conduit and cause the emboli. This event leads to a loss of xylem conductance (Tyree and Zimmermann, 2002). The tension causing cavitation and embolism is differed among species; it might rank from value close to zero such as −0.04 MPa which was found on *Celastrus orbiculatus* (Tibbetts and Ewers, 2000) to as low as −14.1 MPa in conifers (Willson *et al.*, 2008). The variation in xylem vulnerability to cavitation was found to correlate with drought tolerance of the species (Cochard *et al.*, 2008; Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003): the xerophytes appear to be less vulnerable to cavitation compared to mesophytes

and hygrophytes. Genetic variability (Cochard *et al*., 2007; Wortemann *et al*., 2011) as well as phenotypic plasticity (Awad *et al.*, 2010; Corcuera *et al.*, 2011; Herbette *et al.*, 2010) were found within species for xylem vulnerability to cavitation. However, the relationship between xylem vulnerability to cavitation and drought resistance is still unclear for this intraspecific level. In some studies (Cochard *et al.*, 2008; Kavanagh *et al.*, 1999; Kolb and Sperry, 1999) a correlation between both traits was found whereas it was not the case for other studies.

Although there are several studies on xylem vulnerability to cavitation within tree species, the variability of this trait for agronomic species remains to be investigated. Indeed, crop species underwent different selection pressure than natural species. Moreover, the effect of selecting for yield on vulnerability to cavitation is still unclear (Cochard *et al.*, 2007; Lamy *et al.*, 2011) as well as the relationship between this trait and drought resistance at the intraspecific level. We carry out our study on three agronomic tree species: walnut, rubber and apple trees. The three species are chosen because they are different in various ways which will certainly allow the opportunity to explore the intra-species variation in xylem vulnerability to cavitation. The populations within the chosen species have different distances of relation between them. The walnut trees used in this study are cultivars: six of commercialized Persian walnuts (*J. regia*) and six hybrid walnuts (*J. regia* x *J. nigra* NG 38). The former are fruit-oriented while the latter are wood-oriented walnuts. Among the six Persian walnuts, they have different parentages and country of origins. The rubber trees included ten commercialized clones used in Thailand. They have closer genetic relation since they are derived from Wickham's rubber populations which are Brazilian rubber seeds, brought back to Kew Botanical Garden by Sir Henry Wickham in 1879. The apple trees are progenies of a cross between 'Starkrimson' and 'Granny Smith'. The three species we investigated have different features with different genetic structures, what increase the likely to find within species variation in vulnerability to cavitation. This assumption is supported by the distribution of the species and the variation in traits correlated to drought tolerance which previously found.

The choice of these three species was also of agronomic interest, since their productions are under constraint of water deficit. The walnut trees, a drought sensitive species, are mainly produced in the marginal areas where severe drought stress is likely to occur more often in the coming future from the impact of climate change. The natural rubber production in Thailand is expanding more and more into non-traditional production areas. The

sustainability of the production in these areas is questioned because of the risk of drought stress which is predicted to amplify in the future because of the increasing evaporative demand. Some apple productions are in the areas which drought stress might intensify in the future but the interest of drought tolerance genotypes is rising mainly from the need to economizing irrigation cost in the production. Hence, this study aims to assess the genetic variability in xylem vulnerability to cavitation for the three agronomic tree species and to assess the relationship with drought resistance. For the latter, Plants were submitted to a drought and different trait responses were analyzed in order to gain more insights on the variability for drought resistance within investigated species.

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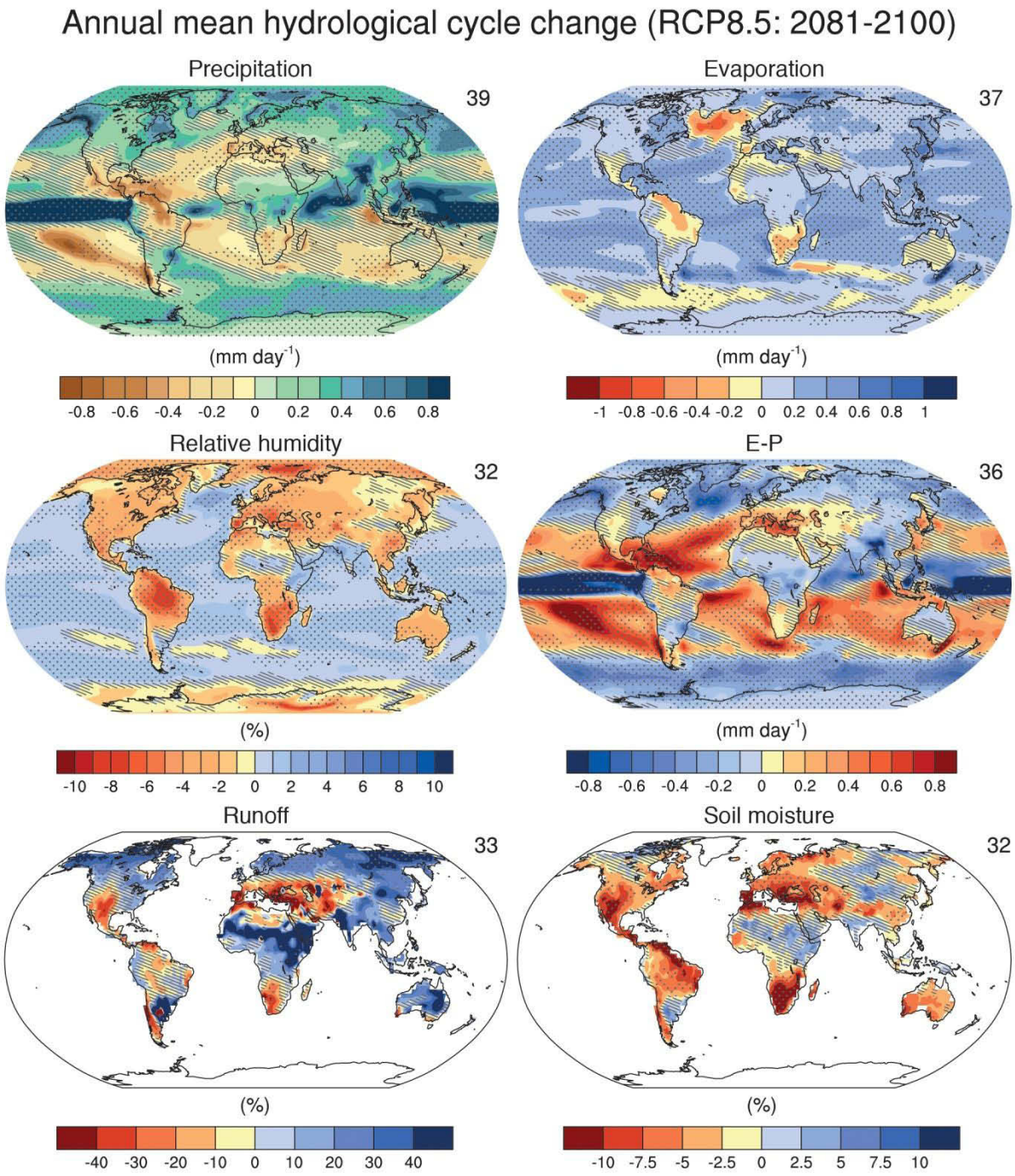


Figure 2. The projection of annual mean changes in precipitation (P), evaporation (E), relative humidity, E – P, runoff and soil moisture for 2081–2100 relative to 1986–2005.

Global maps show multi-model means of hydrological cycle changes. The means are calculated from the Coupled Model Intercomparison Project Phase 5 (CMIP5), the number of models used is indicated in the upper right corner of each panel. Hatching indicates regions where the multi-model mean change is less than one standard deviation of internal variability. Stippling indicates regions where the multi-model mean change is greater than two standard deviations of internal variability and where 90% of models agree on the sign of change. From IPCC (2013).

I. Drought stress

Drought could be defined as a period of time in which water is limited for plant causing a depletion of plant water. When the amount of water loss in plant exceeds the amount of water that plant could acquire, drought stress occurs. Soil dehydration, high evaporation, osmotic binding of water in saline soils or in frozen soil are examples of the causes of drought stress. The severity of drought stress depends on many factors such as occurrence and distribution of rainfall, evaporative demand and moisture storing capacity of soil.

Changes in climate and hydrological cycle are predicted for the future (IPCC, 2013). Although the degree of changes in these climate and hydrological factors are not consistent for all the regions, the increasing dryness in the Mediterranean, the south-western USA and southern African is very likely to occur (Figure 2). The decrease in soil moisture predicted for large regions over the world augments the risk of drought stress in coming years (IPCC, 2013). The major trend of changes in climatic conditions that appear to increase the frequency and severity of water deficit are the globally rising surface temperature and the decreasing precipitation in some regions.

1. Effects of drought stress

Drought stress could affect various biological processes in plant depending on duration and severity of the stress. At cellular level, water deficit could result in loss of turgor pressure, changes in plasma membrane composition and fluidity, changes in water activity and/or solute concentration, and interaction between protein and lipid or between proteins. Water depletion from cell disturbs the bilayer structure of membrane, resulting in the deformation and the increase in membrane porosity. It might also cause the displacement of membrane proteins which cause the loss of enzyme activities and membrane's selectivity. Cellular metabolism could also be disturbed by the accumulation of electrolytes from the dehydration of protoplasm (Mahajan and Tuteja, 2005).

On the whole plant level, drought stress could disturb plant growth and development and thus it could reduce the crop yield. In addition, it could be a cause of plant mortality when severe drought stress occurs. In nature, drought stress from changes in climatic conditions has been reported to associate with mortality and shifts in ecosystem (Allen *et al.*, 2010). The climatic-

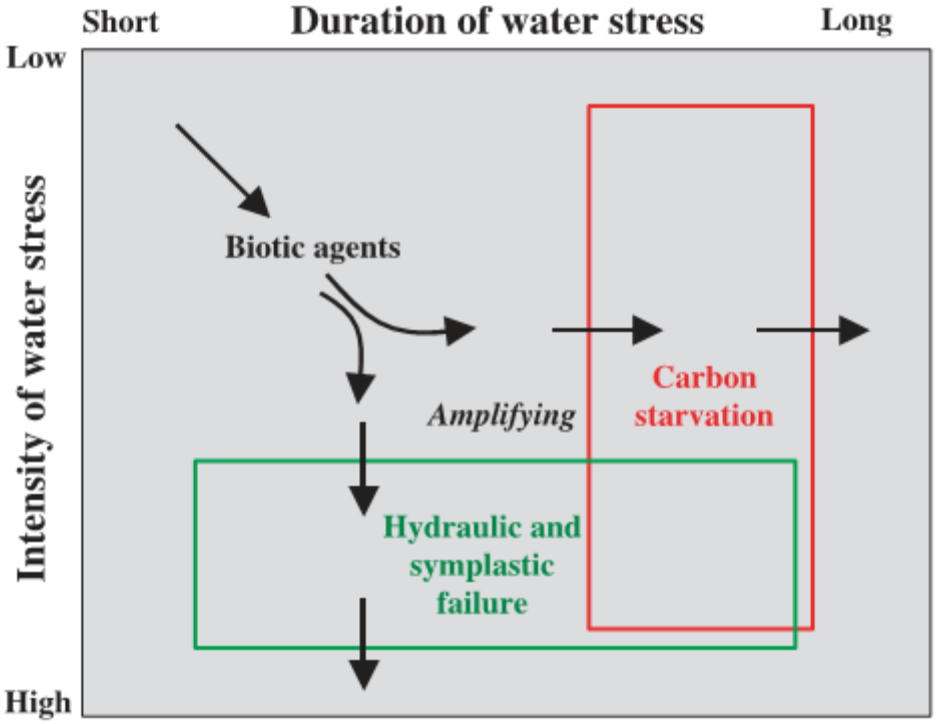


Figure 3. The hypotheses on drought-related mortality mechanisms.

The relationships are based on hydraulic framework of the intensity and the duration of water deficit. Three mechanisms: biotic agents, hydraulic and symplastic failure and carbon starvation are regarded as the main causes of plant mortality. The biotic agents such as pathogens and insects could intensify or be intensified by the other two mechanisms. The failure in water transport is expected to occur when water stress is intensified whereas the carbon starvation is hypothesized to happen with lengthen duration of drought stress. From McDowell *et al.* (2008).

related vegetation mortality is found in various regions over the world such as in the Australian forest (Rice *et al.*, 2004), the tropical moist forests in Indonesia (Van Nieuwstadt and Sheil, 2005), the Amazonian rainforest (Malhi *et al*., 2009), forest of the south-western North America (Breshears *et al.*, 2005) and the Mediterranean and Europe areas (Bigler *et al.*, 2006; Martínez-Vilalta *et al.*, 2002).

According to McDowell *et al.* (2008), the main causes of drought-induced mortality included the biotic agents such as pathogens and insects, the hydraulic failure and the carbon starvation. The relative contribution of these threats depends on the intensity and the duration of stress (Figure 3). When soil cannot supplies sufficient water to compensate water loss from plant, the failure of water transportation system is predicted to occur from xylem cavitation and embolism (see section on vulnerability to cavitation). This could further prevent water supply, cause the desiccation of plant tissues and result in plant mortality. This mechanism is likely to occur if the intensity of drought stress is high. The carbon starvation mechanism is likely to occur when the duration of drought stress is long but the intensity of drought is not high enough to cause the hydraulic failure. Stomatal closure happens early in order to prevent the hydraulic failure in plant when facing water stress. This stomatal closure limits carbon uptake and thus plant photosynthesis. While the stomata are closed, plant still continues to metabolize and its carbon reserves might be used for the metabolism. If stomatal closure and limited photosynthesis are prolonged, it could result in the inadequate carbon reserves and the carbon starvation. The biotic agents are considered as amplifying factors for hydraulic failure and carbon starvation; conversely they are amplified by the other two mechanisms.

For agriculture, the main concerns of drought effect are the reductions in growth and yield. As the quality and quantity of plant growth depend on cell division, cell elongation and cell expansion (that required the turgor pressure in the cells), water deficit affects these processes (Nonami, 1998). However, the survival is also important, especially for the perennial species like trees. Decreased in biomass production is caused by the reduction in photosynthesis. During drought stress, stomata close to conserve water. This regulation is limiting the pathway for gas exchange and it is also causing leaf temperature to increase; both result in the reduction of photosynthesis (McDowell *et al.*, 2008; Shao *et al.*, 2008).

The threat of drought stress on agriculture is highlighted by predicted climate changes and the food security issue as world population is increasing. There are differences in vulnerability to drought stress between regions (Maliva and Missimer, 2012); some might be more vulnerable because of the low gap between average water use and the safe yield of a system and the less adaptive capacity of the water system and society. For example in some African countries; the resources in the normal year might barely sufficient or even insufficient to meet the local needs, the possibility to overcome multiple-year drought stress of these countries could be very thin. It is thus necessary to improve water use efficiency in agriculture which will require the integrative approaches of water resource management, development of drought tolerance crop varieties and also the strategy to meet the global food demand with less water.

2. Defense mechanisms against water deficit

Species respond differently to drought stress, it depends on various morphological, biochemical and physiological responses. Some species could withstand higher level of water deficit and/ or survive longer period of drought stress than others (Klos *et al.*, 2009; McDowell *et al.*, 2008; Mueller *et al.*, 2005). These differences are the results of defense mechanisms against water deficit adopted by the species. The mechanisms to cope with drought stress are categorized into drought escape, desiccation tolerance and desiccation avoidance strategies (Levitt, 1980). However, these strategies are not exclusive and plants might develop a range of response types (Ludlow, 1989).

Plants that escape drought stress are able to grow and reproduce when there is still sufficient moisture. This strategy is found in annual species, especially in arid regions. The species might combine the short life cycle with high growth rate and gas exchange during the short period of moisture (Maroco *et al.*, 2000). The success of drought escape depends on the phenology of plants that matched with periods of soil moisture availability.

Unlike the annuals, perennial species cannot use the escape as a strategy to cope with drought stress because of their long life cycle; hence other strategies are adopted that I develop in the two following sections.

2.1 *Drought avoidance mechanisms*

The avoidance mechanisms include the responses that avoiding tissue dehydration while maintaining the water potential as high as possible. This includes plant responses to minimize

water loss and to maximize water uptake in order to avoid the stress conditions. On one hand, water loss could be minimized by stomatal closure, decrease transpirational area through leaf growth inhibition and leaf shedding, reduce light absorbance by leaf rolling, increase trichome layer density, or change the angle of leaves. On the other hand, water uptake could be maximized by reallocation of nutrients to increase root exploration at the expense of shoot development (Chaves *et al.*, 2003). The water uptake could also archive by increasing root absorption via the activation of aquaporines (Javot and Maurel, 2002).

Stomatal control is a short term response of plant to water deficit; stomatal closure prevents further loss of water from the transpiration and thus maintains or increases the water potentials in plant. The stomatal regulation in response to water deficit could be categorized into two behaviors according to the continuum of the stomatal functioning: isohydric and anisohydric behaviors (Tardieu and Simonneau, 1998). Plants having an isohydric behavior close their stomata in response to decreasing soil water potential and increasing transpirational demand. The stomatal closure will maintain leaf water potential at a relatively constant value despite drought conditions. In contrast, the anisohydric plants keep their stomata open and allow decrement in their leaf water potential as drought progress. Actually, difference between these two theoretical behaviors is not so clear, and all situations could not be classified in only two sets. Isohydric and anisohydric behaviors are rather to be viewed as the extreme situations in a continuum of various plant strategies.

Leaf shedding could be considered as a mechanism of plant to avoid further loss of water but on the other hand, it could also be seen a result from failure of stomatal regulation to prevent xylem embolism. Leaf shedding occurs later than other processes and it is mostly found when plant is facing to a severe drought stress. It allows plants to protect critical organs ensuring survival, that is to say keep buds from dehydration.

2.2 *Drought tolerance mechanism*

Desiccation tolerance involves the ability of plant to keep their metabolism functioning under low water potential. The main drought tolerance mechanisms are: osmotic adjustment, antioxidant systems and resistance to xylem cavitation.

Osmotic adjustment is considered a critical adaptation process of plant to drought stress; it maintains metabolic activities and helps plant to recover after drought stress. When facing drought stress, many plants accumulate compatible solutes such as proline, mannitol, and

glycine betaine (Chen and Murata, 2002). This accumulation results in an increase in cellular osmolarity and leads to an influx of water into cells (Hare *et al.*, 1998). This process could be largely varied between plants. However, the osmotic adjustment is not necessary to have a consistent benefit with yield since cell turgor maintenance is often associated with slow growth (Serraj and Sinclair, 2002).

The capacity to establish the antioxidant protective mechanisms during drought stress and to maintain systems during the rehydration are key factors to the plant recovery (Kranner *et al.*, 2002). During drought stress, the imbalance between light capture and its utilization because of the photosynthesis inhibition leads to an over-excitation of the photosystem II, with a lack of electron acceptor. The resulting excess of electrons from the photosystem II increases the accumulation of superoxide and hydrogen peroxide (reactive oxygen species, ROS) which could damage membrane lipids and proteins. The induction of ROS scavenging enzymes, such as glutathione reductase, ascorbate peroxidase, and dehydroascorbate reductase, could remove the ROS and prevent the damage from this oxidative stress (Sgherri *et al.*, 1994). The efficiency of the antioxidant system is thus a necessary protective system for the plant to overcome drought stress.

The resistance to xylem cavitation is considered an important trait for desiccation tolerance of woody species. It is a promising criterion for drought tolerance screening due to the current reliable and fast analytical techniques (Cochard *et al.*, 2007; Cochard *et al.*, 2008). Contrary to the osmotic adjustment and the antioxidant system that have been subjected to a huge number of investigations, knowledge is scarce on the relationship between resistance to cavitation and drought tolerance. This is why I conducted the present research. The state of art on vulnerability to cavitation is developed in a section devoted to the water properties of the plant, in the following lines.

II. Water properties and hydraulic architecture

Water has many important functions in plant: it is a solvent for various substances and helps to transport nutrients and hormones across plant's organs, it is used to maintain turgor pressure in cell and driven cell's expansion, and it is a substrate for photosynthesis and it also helps to regulate temperature in plant. Water transportation in plant from soil to leaves is possible because of various specific properties of water. This section describes these hydric and hydraulic properties of the plant.

1. Water properties

Water molecules are polar due to the differences of charge on the molecule; the oxygen atom has a higher electro-negativity while the hydrogen atoms are slightly positive. The differences in charge of water molecules cause them to attracted to each other (cohesive) and to other polar molecules (adhesive). A water molecule could form a maximum of four intermolecular hydrogen bonds. Although these hydrogen bonds are relatively weak bonding forces compared to the covalent bonds, they are responsible for number of the physical properties of water.

Among liquids, water has the highest latent heat of vaporization or heat of fusion (44 kJ. $mmol^{-1}$) meaning that the changing phase of water from liquid to gas requires high energy. This is due to the hydrogen bonds between water molecules. The required energy to achieve the changing phase is removed from the liquid and hence cooled it down. This process is called the evaporative cooling which is critical to the temperature maintaining in plant.

In the water body, each molecule is pulled equally in every direction by neighboring liquid molecules. However, at the interface of water to other mediums such as air, the molecules at the surface are only pulled inward because the lack of cohesion force with the other mediums. This creates an internal pressure and this forces water surface to contract to minimal area, hence a surface tension. Surface tension is a measurement of the amount of force required to break this skin on the surface of water.

The surface tension and adhesion force are responsible for a capillary action which occurs when water climbs upward through a small space such as xylem conduits. In plants, the capillary force helps to suspend water inside the xylem while, in the soil, capillary action tends to move water upward between the soil particles.

2. Water potentials

Water potential is generally measured as a pressure by using the unit bar or mega Pascal (MPa). In plant, it is determined by the force required to move water to a state of free water which could be express as following equation:

$$\psi = \psi_p + \psi_\pi + \psi_m + \psi_g \qquad (1)$$

where Ψ_p, Ψ_π, Ψ_m and Ψ_g are hydrostatic, osmotic, matrix and gravitational potentials respectively.

The hydrostatic potential (Ψ_p) is considered as the effect of pressure in the given plant organ: in cell, this potential is equivalent to turgor pressure which is a positive value whereas it is negative in xylem conduits.

The osmotic potential (Ψ_π) is defined as the potential of water molecules to move between regions of differing concentrations across a permeable membrane. This movement depends on the concentration of solutes (C), the universal gas constant (R, 8.314 $J.K^{-1}$ mol^{-1}) and the absolute temperature (T, Kelvin) as following equation:

$$\psi_\pi = -CRT \qquad (2)$$

The osmotic potential of pure water is zero while water with solutes has a negative potential.

The matrix potential (Ψ_m) quantifies tendency of water to adhere to surface (surface tension), it is mostly important for determining soil water potential whereas it could be neglected in living cells of plant.

The gravitational potential (Ψ_g) defines as energy used to move object up to a fixed reference location; for water column in plant, Ψ_g is decreased by 0.10 MPa every increasing 10 m above the reference location.

Plant water potential is an intermediate value between the soil and atmospheric water potential. It is a dynamic value which could vary according to various factors such as organs of plant, position of the organ on the tree or time of the day. The water potential of root (Ψ_{root}) is more negative than Ψ_{soil} because of higher ion concentration in root cells; water could enter the

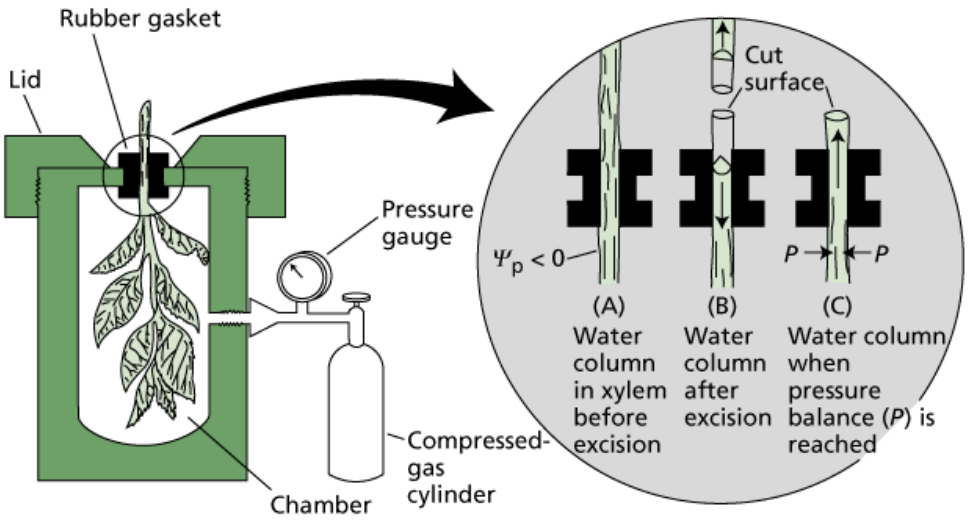


Figure 4. The pressure chamber used for measuring plant water potential.

The left figure shows pressure chamber diagram with a shoot sealed into it. The chamber is pressurized with compressed gas. When the shoot is still intact, water column is under tension (A) but when the shoot is cut water column is pulled back into the tissue in response to water tension in the xylem (B). By applying pressure inside the chamber, water in the shoot is forced to re-emerge at the cut surface (C). The pressure required to bring back water to the cut surface is equal to the tension in xylem. From Taiz and Zeiger (2010).

cells by the reverse-osmosis. The leaf water potential (Ψ_{leaf}) varies daily with two extreme values. The highest value, Ψ_{pd} (maximum leaf water potential), is measured before the dawn.

Indeed, Ψ_{pd} reach a value near those of the soil water potential after a long night without transpiration. This Ψ_{pd} allow thus evaluating the water potential of the soil. The lowest leaf water potential (Ψ_{md}, minimum leaf water potential) is the water potential measured at midday, when leaf is heavily transpired and the water loss is thus maximal. The estimation of water potentials in plant organs such as leaves or shoots is done with the pressure chamber (Figure 4) (Scholander *et al.*, 1965).

3. The ascent of water

The ascent of water in plant (Figure 5) is explained by the "cohesion-tension" theory (Dixon and Joly, 1895). At the evaporation surfaces, in stomatal cavity, water is suspended to tiny meniscuses. When water evaporated, the radius of these meniscuses tends to decrease into the conduits. This process increases conduit's capillary forces and generates negative hydrostatic pressure (tension) that transmits through the adjacent water molecule. According to Young-Laplace law, the tension (ΔP) is described as following equation:

$$\Delta P = \frac{-2\gamma}{r} \qquad (3)$$

when γ is water–air surface tension and r is radius of curve meniscus. Since water molecules are very cohesive, this tension pulls whole water columns in xylem upward to compensate evaporated water. Therefore, water flow in plant could be considered as a continuous system from soil into plant and out to atmosphere (Soil-Plant-Atmospheric Continuum, SPAC). The flow (F, $kg.s^{-1}$) is described as following function:

$$F = K \times \Delta\psi \qquad (4)$$

where K is hydraulic conductance ($kg\ s^{-1}\ MPa^{-1}$) and $\Delta\Psi$ (MPa) is the gradient of water potential across the system.

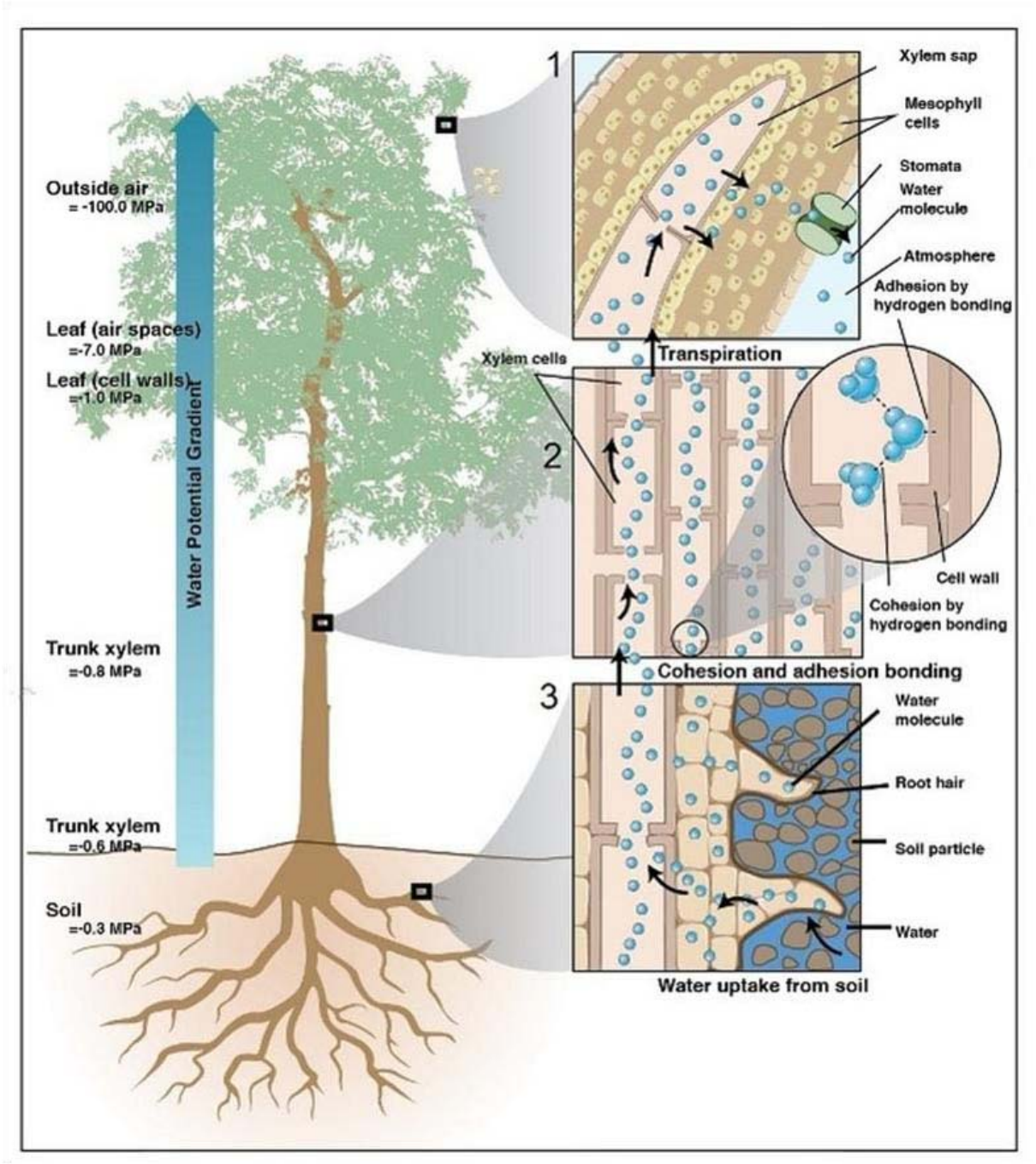


Figure 5. The ascent of water in tree according to the tension-cohesion mechanism.

Water is absorbed along a gradient of decreasing water potentials, from soil into root then transported in xylem conduits toward leaves where the evaporation occurred. Motor of this ascent is the evaporative force due to the very low atmospheric water potential. From McElrone *et al.* (2013).

3.1 *Water absorption and soil-root boundary*

The water molecules are retained between the soil particles in various pore sizes. In very wet soil, the soil water potential (Ψ_{soil}) is close to the water potential of pure water at atmospheric pressure (close to nil). After gravitational percolation, the water kept in soil is the maximum water content which is called field capacity. Plant absorbs water from the soil into plant by roots that are in contacted with water in the soil. This absorption is functioning as long as the Ψ_{root} is more negative than Ψ_{soil}. The Ψ_{soil} becomes more negative when soil dried out due to the increasing surface tension between water and air. This could limit water transport to tree roots. The hydraulic conductance of root which is an inverse value of root resistance is positively linked to the spatial extension and the density of their root system. Fine root exchange surface is crucial for the effectiveness of water extraction; the tree with deep, well-distributed and dense root system is considered to be more efficient as these traits allow tree with better access to soil water reserves as well as nutrients (Levitt, 1980). Once absorbed, water crosses root cells through 2 main pathways: apoplastic and symplastic (Figure 6*A*). In the former pathway, water moves between root cells without crossing plasma membrane. In the latter pathway, water travels through cytoplasm of root cells, passing from cell to cell via plasmodesmata; it could also cross the plasma membrane. At root cortex, the endodermis cells are water impermeable due to the lignified casparian bands, hence, water must enter the symplastic pathway after crossing the plasma membrane. Here, aquaporines are expected to play a critical role in root hydraulic conductance, especially during water stress (Aroca *et al*., 2012).

3.2 *Transpiration and stomatal control*

Once water reaches xylem conduits, it travels upward to leaves through these conduits which are considered a part of the apoplastic pathway. At the leaf, the gradient of water potential pulls water from the xylem conduits into the leaf mesophyll cells. Water molecules evaporate into the inter-cellular space in leaf and then exit to the atmosphere.

The leaves of land plants are covered with cuticle, a waxy layer, which prevents water loss and gas exchange from these surface. Both water loss and gas exchange occur through the stomatal opening (Figure 6*B*). The pair of guard cells surrounding the opening regulates water loss from transpiration as well as the uptake of carbon dioxide and release of oxygen by closing or opening the stoma. The water potential of the air (Ψ_{air}) is almost always strongly

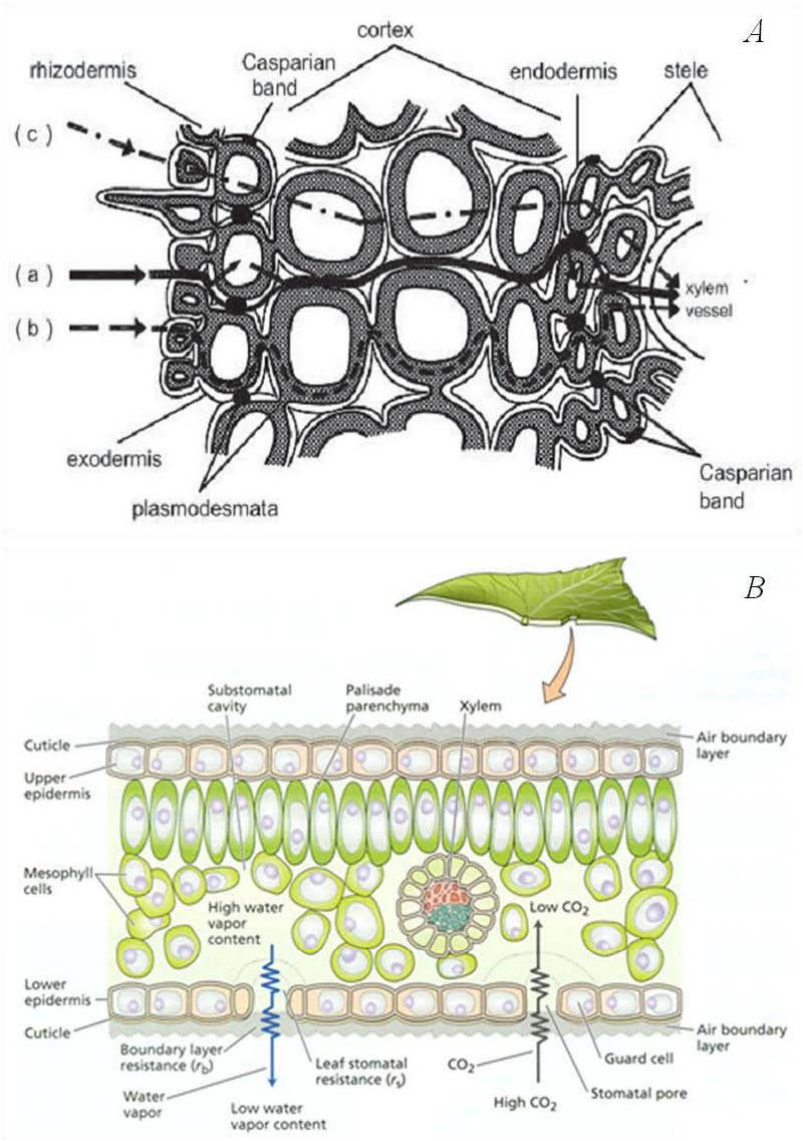


Figure 6. Water and ions transportation in root (*A*) and gas exchanges at leaf (*B*).

Water transportation in root (*A*): water crosses root cells through (a) the apoplastic pathway in which water moves between root cells without crossing the plasma membrane, (b) the symplastic pathway in which water flows through cytoplasm, it crosses the plasma membrane and passes from cell to cell through plasmodesmata. While the pathway (c) is the transcellular pathway which water flows across membranes. At leaf, transpiration and gas exchanges occur through stomatal opening (*B*): the blue arrow shows the vapor moves out of leaf while CO_2 diffuses into leaf is indicated with gray arrow (Taiz and Zeiger, 2010).

negative. The actual value depends on the absolute temperature and the relative humidity of the air. The Ψ_{air} could be calculated as:

$$\psi_{air} = \frac{RT}{V_m} In \frac{e}{e_0} \quad (5)$$

where R is the molar gas constant (8.31 J.mol^{-1}K^{-1}), T is the dry bulb temperature in Kelvin, $\frac{e}{e_0}$ is air relative humidity and V_m is the partial molar volume of water (18.0 × 10^{-6} m^3 mol^{-1}). For example, the air with 70% relative humidity could have its water potential ranking from −46.56 to −49.65 MPa when air temperature is between 10 to 30 ˚C.

The majority of water flow through plant is transpired while another small part is used as a substrate for photosynthesis. The loss of water via transpiration is a necessity for the uptake of water and nutrients from soil and it is also an important mechanism for the temperature control in plant.

Because Stomata control plant transpiration (E) and gas exchanges, it prevents excessive loss of water from plant and also plays an important role to keep the balance between water loss and carbon uptake. During the day, stomatal closure occurs even under non-restricting soil water availability indicating a diurnal circadian rhythm behavior (Gorton *et al.*, 1993; Mencuccini *et al.*, 2000). The degree of this regulation depends on several factors such as air humidity, temperature and water status of plant. The simple interaction of stomatal regulation (g_s) is provided (Whitehead, 1998) as following equation without taking into consideration of water storage:

$$E = g_s \times VPD \times A_l = g_s \times (\psi_{soil} - \psi_{leaf}) \times A_{sw} \quad (6)$$

where VPD is water pressure deficit, A_l is leaf area and A_{sw} is sap wood cross sectional area.

Studies found that the decrement in xylem water potential during the onset of drought stress and leaf water potential could trigger stomatal closure (Cochard *et al.*, 2002; Hubbard *et al.*, 2001; Nardini *et al.*, 2001). This response is believed to prevent drought-induced cavitation and runaway air embolism in xylem (Cruiziat *et al.*, 2002). Stomatal closure has been reported to occur during soil drying before any change in leaf water potential (Nardini and Salleo, 2000). It is considered a result of chemical signaling, the abscisic acid (ABA),

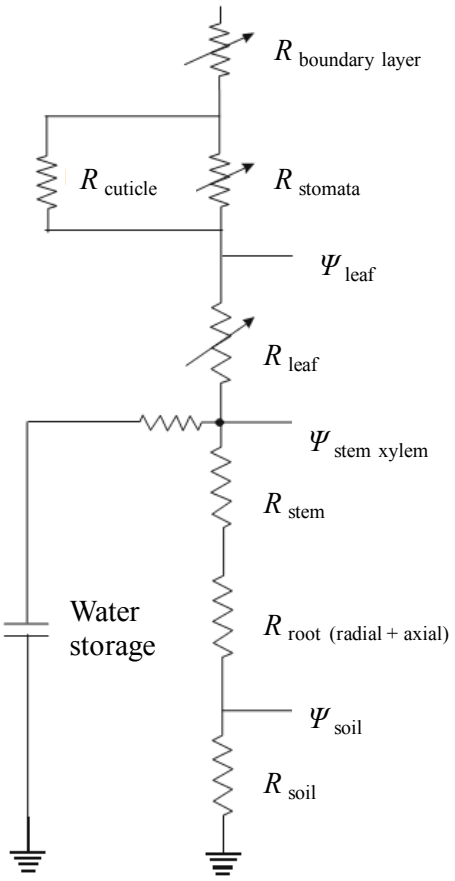


Figure 7. Water flow in the soil-plant-atmospheric continuum (SPAC) according to an Ohm's law analogy.

The Ψ represents water potential in different sections of the plant while the R represents hydraulic resistance. Arrows on resistance icons represent variable resistances; however, other resistances are not absolutely static under all conditions. From Blum (2010).

between roots and shoots. Dehydration of the mesophyll cells triggers the release of ABA stored in the chloroplast and it increases the rate of ABA biosynthesis in mesophyll cells. The accumulated ABA triggers stomatal closure in guard cells by activating calcium- (Ca^{2+}), potassium- (K^+) and anion channels (Leung and Giraudat, 1998).

In order to follow the stomatal regulation, stomatal conductance (g_s, mmol m^{-2} s^{-1}) is measured. It is the rate of passage of water vapor exiting or CO_2 entering through the leaf stomata. The stomatal conductance, or its inverse the stomatal resistance, is directly related to the boundary layer resistance of the leaf and the absolute concentration gradient of water vapor from the leaf to the atmosphere. It is under direct biological control of the leaf through the turgor of guard cells. Stomatal conductance is used as a reference parameter to assess the degree of water stress of species. It is strongly correlated with several photosynthetic parameters and is reported to be under genetic control (Percy *et al.*, 1996). Therefore, it is considered a useful tool for yield improvement in drought-prone environments because it provides crucial information on plant water use efficiency (Jones *et al.*, 1983).

3.3 *Hydraulic properties in xylem: conductance or resistance*

The flow of water in plant is determining from a conductance (K), or a resistance ($R = 1/K$). The hydraulic conductance (K, mmol s^{-1} MPa^{-1}) of the conduit is calculated as ratio of conduit's diameter (D) and $\Delta\Psi$ between two points of the flow:

$$K = \frac{D}{\Delta\psi} \qquad (7)$$

The conductance could also be expressed in a unit of the measured segment's length (L, m) as a hydraulic conductivity (K_h, mmol m s^{-1} MPa^{-1}):

$$K_h = \frac{D}{\Delta\psi} \times L \qquad (8)$$

The specific conductivity (K_s, mmol m^{-1} s^{-1} MPa^{-1}), a hydraulic conductance of a plant's segment such as branch or petiole as a function of length and conductive surface on the wood (A_s, m^2), is calculated as:

$$K_s = \frac{D \times L}{\Delta\psi \times A_s} = \frac{K_h}{A_s} \qquad (9)$$

Under steady state conditions, water flow in a soil-plant-atmosphere continuum (SPAC) could be described according to an analogy of Ohm's law (Figure 7) as follows:

$$Flow = \frac{\psi_{soil} - \psi_{root}}{R_{matrix}} = \frac{\psi_{root} - \psi_{leaf}}{R_{root} - R_{xylem}} = \frac{\psi_{leaf} - \psi_{air}}{R_{stomata} - R_{air}} \quad (10)$$

where, R_{matrix} is the resistance due to the soil matrix, R_{root} is the root resistance, R_{xylem} is the resistance through the xylem in plant stems, $R_{stomata}$ is the stomatal resistance, and R_{air} is the aerial resistance. In well hydrated plant, the resistance of leaf represents the majority of whole plant resistance which might rank from 26 to 89% depended on the species (Sack and Holbrook, 2006).

The R_{xylem} in stem are, generally, the lowest; the total R_{xylem} is a combination of conduit lumen and interconduit pit resistances (R_{lumen} and R_{pit}, respectively). The R_{lumen} could be calculated according to Hagen-Poiseuille equation as:

$$R_{lumen} = \frac{128\eta L}{\pi D^4} \quad (11)$$

where L and D are length and diameter of conduit, respectively. Although pits allow the passage of water between xylem conduits, they constitute a limiting factor for water flow in xylem (Comstock and Sperry, 2000). The resistance of pit (R_{pit}) is determined by the area-specific resistance (r_p) and the surface area of overlap between conduits (A_{pit}):

$$R_{pit} = \frac{r_p}{A_{pit}} \quad (12)$$

The R_{pit} could be significantly varied across the species but it usually account for 50% or more of the total hydraulic resistance in the xylem (Choat *et al.*, 2008).

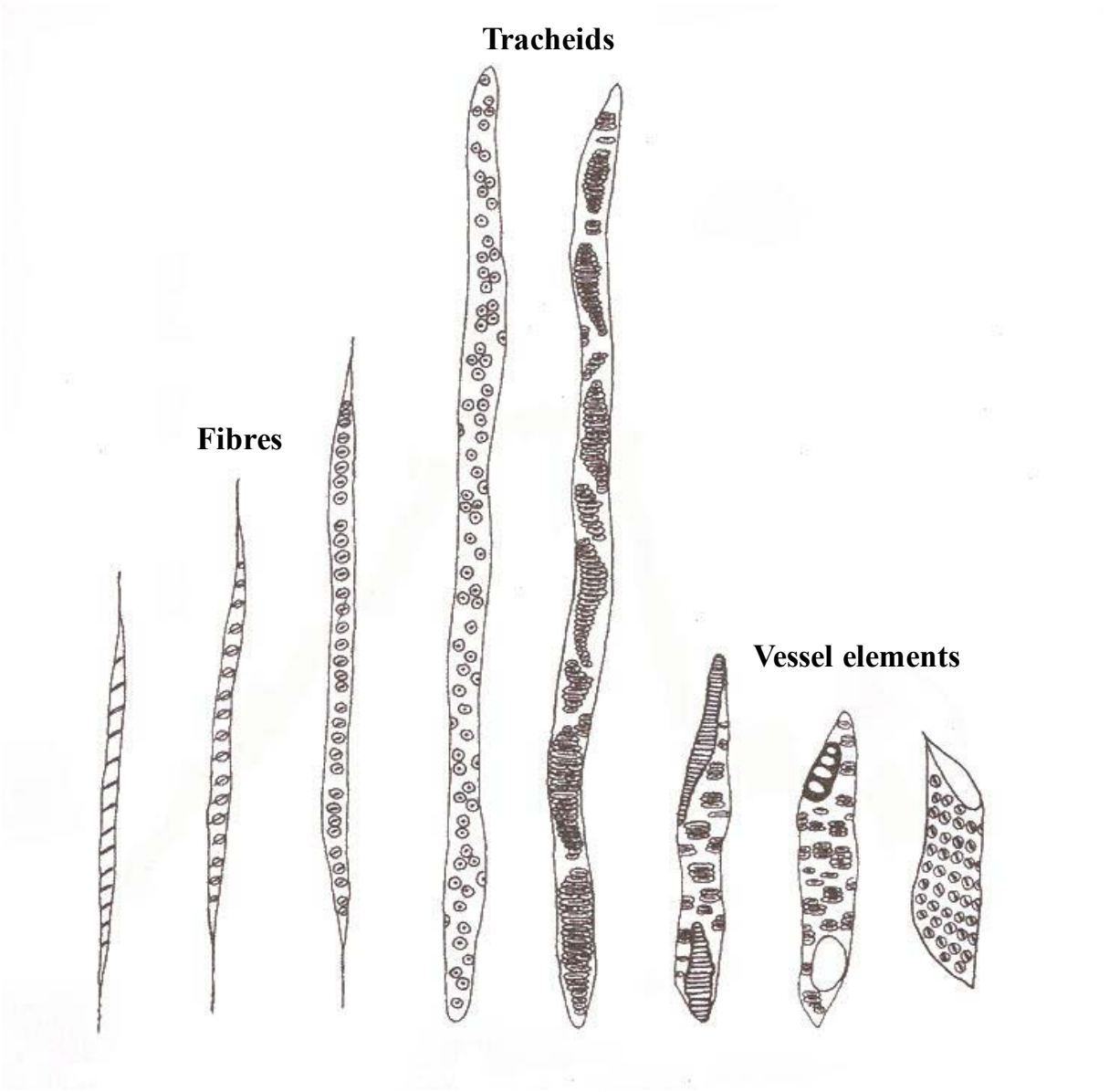


Figure 8. Relative sizes and shapes of xylem sclerenchyma cells in mature wood of some angiosperms and gymnosperms.

Drawings show different types of sclerenchyma cells: fibers, tracheids and vessel elements. In angiosperms, the tracheary elements are vessel elements and tracheids and they are specialized for water transport while fibers provide mechanical rigidity and support to plants. In gymnosperms, tracheids are not only conductive but they also provide support for the trees. Figure is modified from Alden (2009).

III. Xylem: cell types and structure

Majority of water transport occurs in a specific pipe-like structure found in the xylem which permits long distance water transportation from roots to leaves. Xylem tissue is composed of different cell types that could be classified in two different types: parenchyma and sclerenchyma cells. The parenchyma cells are responsible for most of the storage function of xylem while sclerenchyma cells, including fibers and tracheary elements (vessel elements and tracheids), are involved in mechanical support and water transportation (Figure 8). Angiosperms and gymnosperms show big difference for sclerenchyma cells: the formers have vessel elements, fibers, parenchyma cells and tracheids are found in some species such as oaks and chestnuts, whereas gymnosperms contain tracheids and small amount of parenchyma cells. The vessel elements in angiosperms are specialized for water transport and fibers provide mechanical support while tracheids in gymnosperms are not only conductive but also provide support for the trees (Myburg *et al.*, 2007).

The mature parenchyma cells serve as carbohydrate reserves; they store starch in their functional protoplasm. These cells could differentiate to form callus and generate functional xylem cells. Parenchyma cells have some part of their wall which consist of thin primary cell wall called primary pit fields. Water and nutrients could pass through them and thus allow cell-to-cell movements. Non-living mature tracheary elements are cells without cellular contents; they play a passive role in the transport of water through the plant. In order to withstand large tension from negative pressure inside the conduits, these cells developed thickened secondary wall impregnated with lignin. Tracheids are connected via large bordered pits which are concentrated around the ends of the cells. Vessel elements are end-to-end connected through their large perforation, and these connected vessel elements form a vessel.

Conduit diameters and lengths are largely varied; the span of diameter covers the magnitude from below 5 µm for conifer needles to over 500 µm for lianas vessel elements (Ewers, 1985) while range of conduit length could be from a few millimeters in tracheids up to several meters as demonstrated in previous studies (Ewers and Fisher, 1989a; Zimmermann and Jeje, 1981).

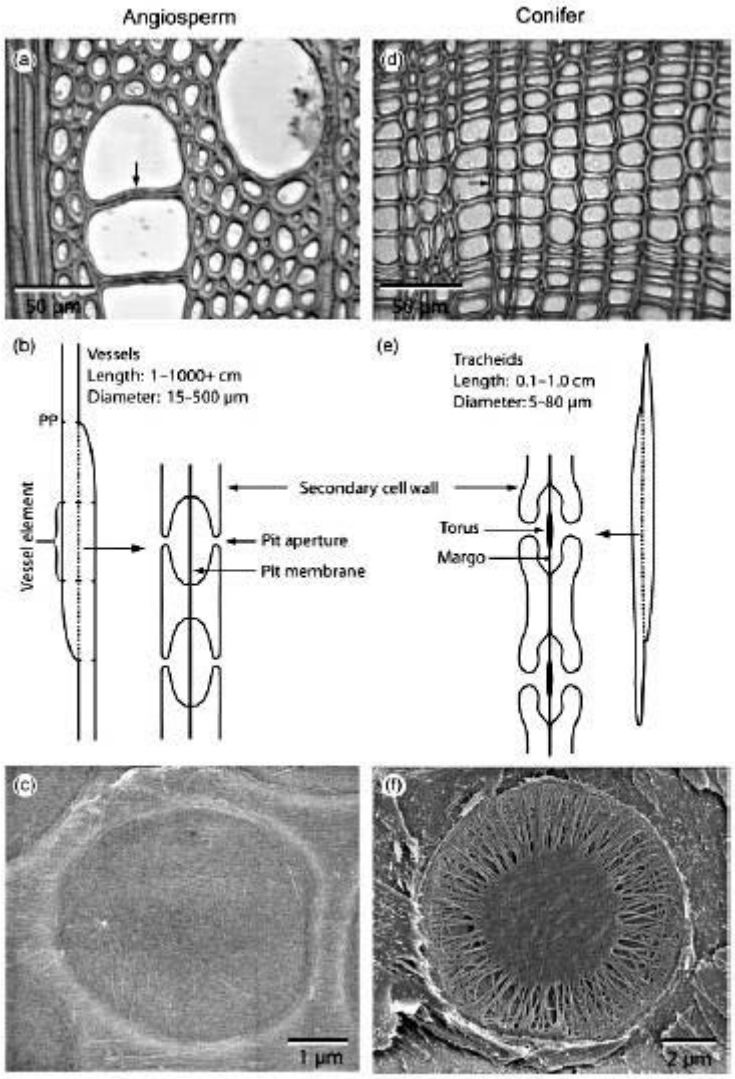


Figure 9. Structure of xylem and interconduit pits in angiosperms (left) and conifers (right).

Figures on the left side show xylem structure and interconduit pits of angiosperm. (a) Transverse section of xylem tissue showing vessels connected through pitted walls. (b) Each vessel is made up of multiple vessel elements joined end-on-end through a perforation plate. Vessels are connected through bordered pit pairs with a pit membrane derived from two primary cell walls and a middle lamella, (c) SEM image showing 'homogeneous' pit membrane with a uniform deposition of microfibrils across the surface of the membrane. Figures on the right side show xylem structure and interconduit pits of conifer. (d) Transverse section of typical conifer xylem tissue made up of tracheids with bordered pits located in radial walls. (e) Tracheids consist of a single tracheary element and are therefore constrained to shorter lengths than vessels. The architecture of bordered pits is similar to that of vessels, with the exception of pit membrane structure. (f) SEM of a typical gymnosperm pit membrane with a central thickening (torus) and very porous outer region (margo) (Choat *et al*., 2008).

Xylem vessels are connected together via pit which are wall structures with no secondary wall (Figure 9). In Angiosperms, pit membranes appear homogenous whereas in conifers, a thickened and lignified central part of pit membrane is found. This structure is called torus; it is usually slightly bigger than the aperture of the pit border and water impermeable. Water could move easily through the margo which is a porous network of cellulose fibrils. These pit membranes allow water and nutrients passing through, while they help filtering pathogens, air and other particles between the connected vessels (Crombie *et al.*, 1985).

Xylem sap is generally under negative pressure. During the event of drought stress, xylem sap in a metastable state might rapidly change to vapor and the conduit could be filled with air. The pressure in this air-filled conduit is raised to atmospheric. Because of the present of pit membranes, the expansion of air is restricted within a conduit and the connected conduits might still be sap-filled. The different xylem pressure between air- and sap-filled conduits could cause a deflection of pit membranes. This deflection might increase the membrane porosity in angiosperms with homogeneous pit membranes (Choat *et al.*, 2004; Sperry and Hacke, 2004). Despite the flexibility of pit membranes, after some cycles of cavitation and refilling (Hacke *et al*., 2001b; Holbrook *et al*., 2001), pit membranes might lose their flexibility and become more vulnerable to air-seeding cavitation (Hacke *et al*., 2001b). This increased vulnerability to cavitation due to embolism and refilling cycle is the phenomenon of cavitation fatigue (Hacke *et al*., 2001b). In gymnosperms, the pressure difference between two tracheids might push a torus to block the apertures of the pit-pair and prevent the movement of air through the pit. This might serve to isolate embolized tracheids and thus prevent the spread of embolisms (Tyree and Sperry, 1989b) to the connected tracheids. Thus, the conifer pits are served as safety valves in the hydraulic system of plants.

Vessel diameter and its distribution are used to characterize wood pattern (Figure 10). In angiosperms, there are two main patterns: ring porous and diffuse porous. The vessels of early wood in ring porous are much larger than in late wood; well-defined zone of the vessels is observed and the transition to the late wood within the same growth ring is abrupt. While in diffuse-porous, diameter of the vessels is rather even throughout the growth ring. Ring porous and diffuse porous species are found different in the length of their vessels (Zimmermann and Jeje, 1981). In general, the ring porous species appeared to have long vessels which could be as long as their length of stem while in the diffuse porous, vessel length could be up to 1 m long.

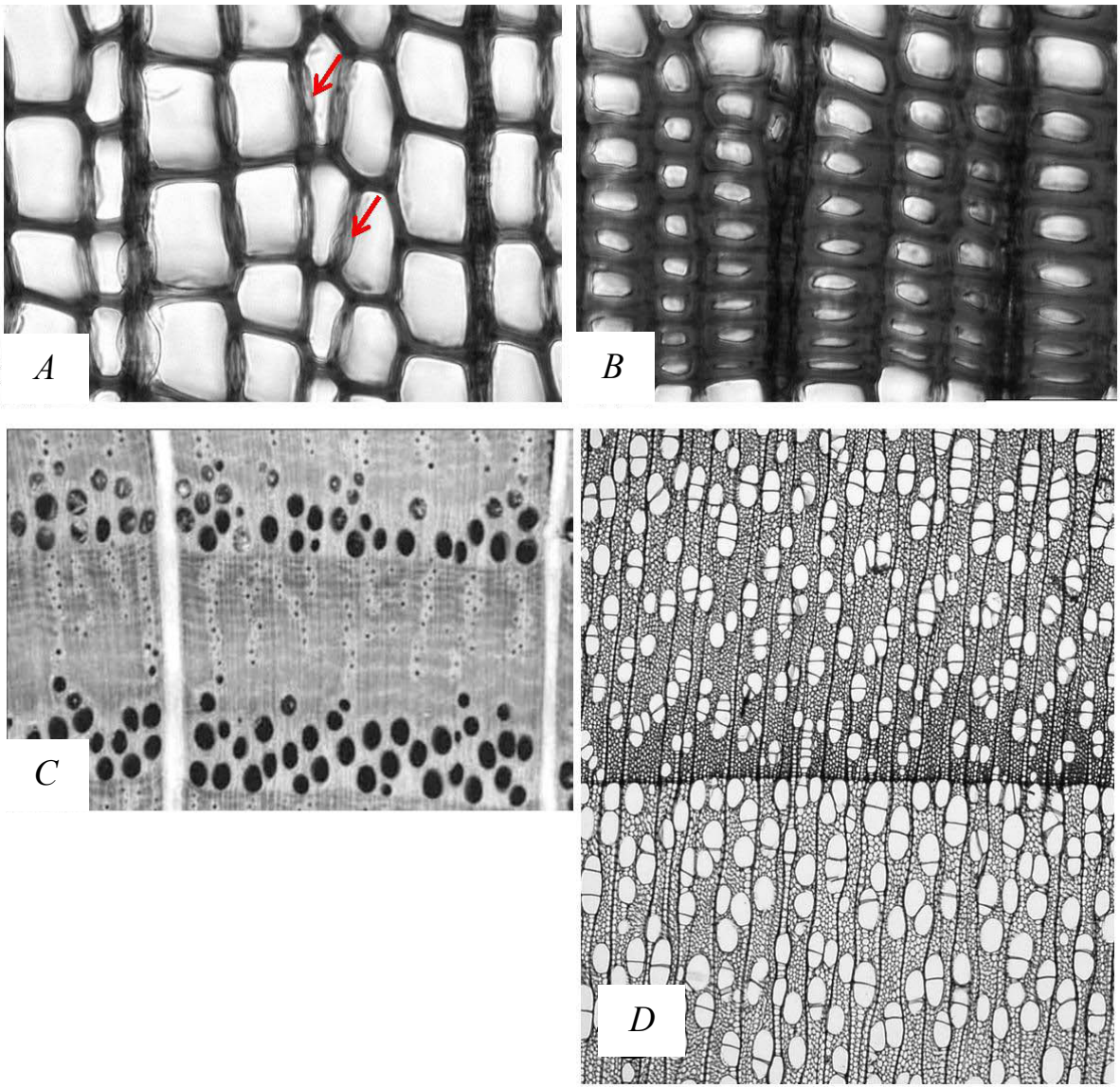


Figure 10. Cross sections of wood view with light microscopes.

Early (*A*) and late (*B*) wood of Scots pine tree (*Pinus sylvestris* L.). The tracheids with large diameter and thin cell walls are presented for the early wood, whereas, smaller diameter and thicker cell walls tracheids are visible in the late wood. The bordered pits on the radial cell walls are marked by arrows. Large early wood vessels are visible (*C*) as a ring close to boundary of the annual increment in the wood of 'ring-porous' oak tree (*Quercus robur* L.). The vessels of silver birch (*Betula pendula* Roth), a diffuse-porous (*D*), are almost the same in diameter within entire growth ring. From Marciszewska and Tulik (2013).

IV. Xylem cavitation and embolism

Water flowing in xylem under tension (negative pressure) is susceptible to cavitation which results as a break of water column in xylem conduits. This situation could occur when tension in conduit is high enough to break the cohesive bond between water molecules (Tyree and Sperry, 1989b). Conduit then becomes air-filled and non conductive (embolized) resulting in reduction of hydraulic conductivity. Water stress and freeze-thaw cycle could initiate cavitation in trees (Sperry and Sullivan, 1992; Tyree and Sperry, 1989b). In this study, we focus on the drought-induced cavitation.

The researches on xylem vulnerability to cavitation were intensively conducted on forest species in the past two decades. The trait is found to vary largely between species and correlate with drought tolerance of the species (Cochard *et al.*, 2008; Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003). It is also varied at the intra-specific level (Corcuera *et al.*, 2011; Herbette *et al.*, 2010; Maherali and DeLucia, 2000; Martínez-Vilalta *et al.*, 2002). Because of these reasons, the vulnerability to cavitation is proposed as a potential criterion for drought tolerance screening (Choat *et al.*, 2012; Maherali and DeLucia, 2000; Matzner *et al.*, 2001). However, the information of this trait is scarce for the agronomic species. Therefore, in this thesis, I chose to investigate the xylem vulnerability to cavitation on three different agronomic species. Following section is devoted to the state of art on this trait.

1. The mechanism of cavitation

In theory, drought-induced cavitation could be explain with different mechanisms: (*i*) a loss of cohesive force between molecules of water in xylem conduits which called homogeneous cavitation, or (*ii*) a loss of adhesive force between water molecule and conduit walls called heterogeneous cavitation (Pickard, 1982). Since the rupture of cohesive force between water molecules is expected to occur only at pressure below −20 MPa (Caupin and Herbert, 2006; Caupin *et al.*, 2012), homogeneous cavitation is unlikely to occur in trees because the required pressure is much lower than the pressure values found in xylem. The cavitation in tree is, therefore, accepted as a result of heterogeneous nucleation in xylem conduits (Tyree *et al.*, 1994).

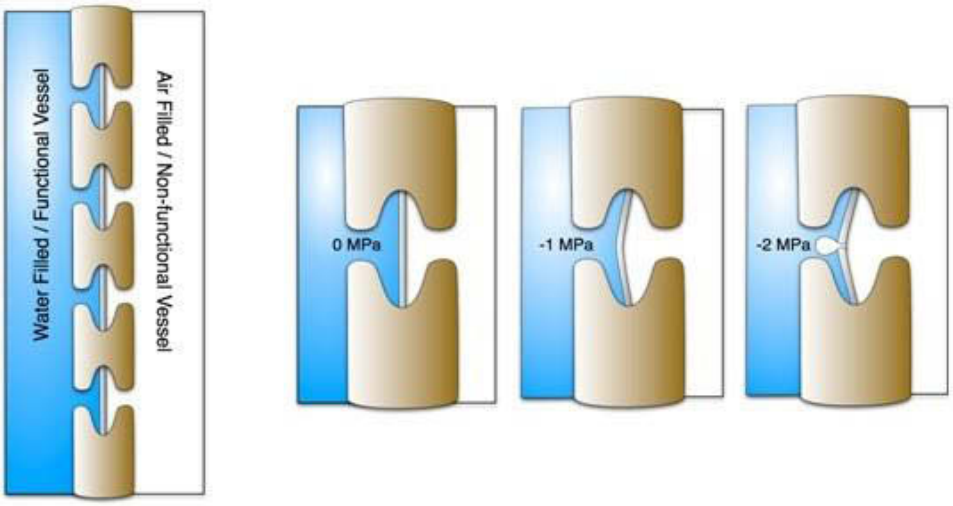


Figure 11. Air-seeding mechanism through pits.

The diagram shows intervessel pit structure and the mechanism of cavitation by air-seeding. When cavitation event occurs in a vessel, air will expand in the vessel and it is stopped at pit membranes. The surface tension of water in the adjacent vessels helps to support pit membranes and the air-water meniscus is held at the opening of each pore. When the pressure difference is exceeded the capillary of air-water meniscus, air is pulled through pit pores. From McElrone *et al.* (2013).

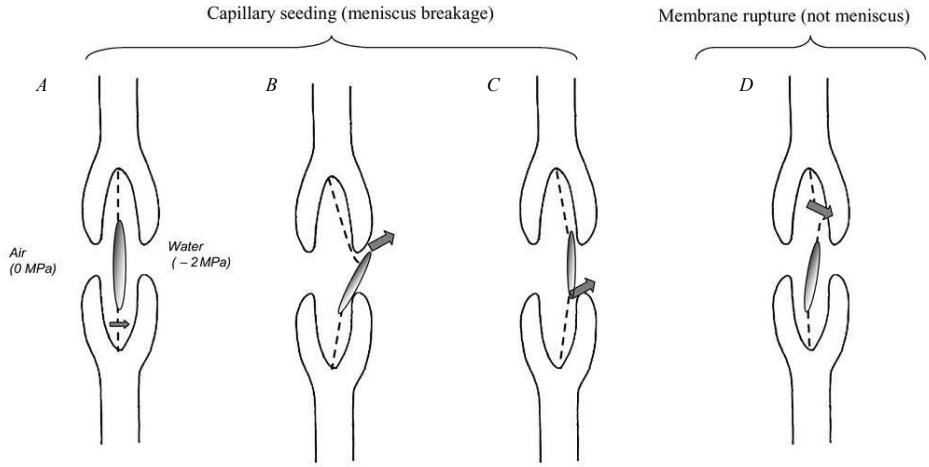


Figure 12. Different hypotheses of air-seeding through a conifer bordered pit membrane.

When the two conduits in contact are water filled, the pit wall lies in the middle of the pit chamber and water flows through the margo. When one conduit cavitated, air could enter the water filled conduit in contact through margo capillary-seeding (*A*), by capillary rupture of an air/water meniscus through pores in the margo when torus aspiration does not occur. Margo stretch-seeding (*B*), occurs by elastic stretching allowing the torus to be pulled out through the pit aperture or through membrane slippage that allows the torus to move off-center. Seal capillary-seeding (*C*), when the torus is not tightly sealed against the pit border (weak aspiration or poor air tightness of torus/pit aperture interface) and (D) margo rupture-seeding, by membrane breakage.

Air could enter plant through the point where xylem conduit is damaged; however, the main source of air in plant is actually the water itself. Plant water is saturated with air at the atmospheric pressure and in the metastable state under xylem tension; it could rapidly change into vapor and fill the conduit. When vessel is cavitated, air will expand in the vessel and it is stopped at pit membranes. The surface tension of water in the adjacent vessels helps to support pit membranes and air bubble is held at the opening of each pore. According to the air-seeding mechanism (Zimmermann, 1983), cavitation occurs when the pressure gradient (ΔP) between xylem water (with negative pressure values) and surrounding air (at atmospheric pressure) exceeds the capillary forces at the air-water interface (Figure 11). Air will be pulled into the conduit and the air bubble will nucleate the phase change to vapor. Then water pressure will rise to atmospheric, allowing the conduit to be drained by the surrounding transpiration stream. The ΔP between vessels exceeds a certain threshold which depends on the radius of the pore in the pit membrane (Tyree and Sperry, 1989b):

$$\Delta P = \frac{2\tau \cos \alpha}{r_m} \qquad (13)$$

where τ is the surface tension of water (0.072 Pa m at 25˚C), α is the contact angle between the meniscus and the pore and r_m is the radius of the meniscus to the radius of the pore in pit membrane.

According to this relationship, pit membrane with larger pores will thus be more vulnerable to air-seeding than pits with smaller pores. This holds for angiosperms; however due to different pit structures, the air-seeding mechanism in gymnosperms is somewhat different. In conifers, following mechanisms are proposed: (*i*) a capillary rupture of an air/water meniscus through pores in the margo without an aspiration of torus (Figure 12*A*); (*ii*) a margo stretch-seeding, the torus to be pulled out through the pit aperture or through membrane slippage that allows the torus to move off-center, exposing a portion of the margo in the pit aperture (Figure 12*B*); (*iii*) a seal capillary-seeding occurs when the torus and the inner wall of the pit membrane are not perfectly sealed, this allows air bubbles to pass through pores at the edge of the torus (Figure 12*C*); and a margo rupture-seeding (Figure 12*D*)which is the rupture in the membrane when low xylem tensions break the fibrils in the margo (Cochard, 2006; Cochard *et al.*, 2009; Pittermann and Sperry, 2006; Sperry and Tyree, 1990).

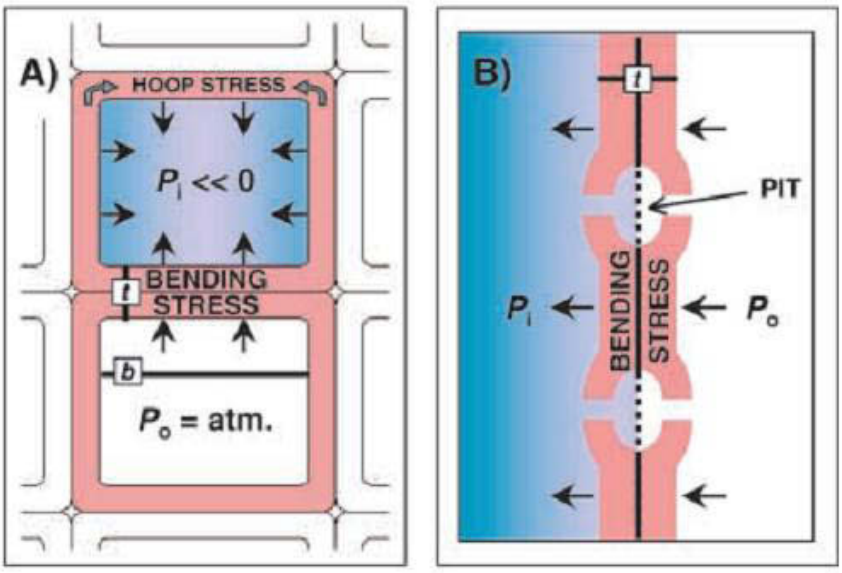


Figure 13. Stresses in the conduit wall arising from negative sap pressure.

Two stresses in a water-filled conduit (shaded): hoop stresses are relatively small while bending stresses which occur in the wall between an embolized- and a water-filled conduits are larger (A). Bending stresses are related to the thickness of the wall (t), its span (b), and the pressure difference, $Pi - Po$, where Po is the gas pressure (near atmospheric) in the embolized conduit and Pi is the pressure of water-filled conduit. Longitudinal view of the wall between water-filled (shaded) and embolized conduits (B); the thickened region bearing the bending stress, and the porous pits through which air-seeding nucleates cavitation. From Hacke *et al.* (2001a).

2. Resistance to xylem implosion and cavitation resistance

Because water in xylem is transported under tension, the structure of xylem has to be strong enough to withstand this tension and prevent a collapse of the conduit wall. The structural traits that control the implosion of xylem involve the transversal mechanical cell wall properties and the shape of the vessel. The thickness of the vessel separating wall (t) and the relative to its maximum span (b) appear to relate with ability of xylem to endure this tension (Awad *et al*., 2010; Hacke *et al*., 2001a; Jacobsen *et al*., 2005) and prevent xylem implosion (Figure 13). Under the negative sap pressure, the conduit wall is under two different constraints: compressive hoop stresses and bending stresses (Hacke *et al*., 2001a). The compressive hoop stresses encircle the wall while bending stresses arise in the wall between sap- and gas-filled conduits. The first stresses are relatively small compared to the latter ones.

To withstand these stresses, the reinforcement ($(t/b)^2$) against collapse from bending is required. The t/b ratio is expected to be able to withstand the lowest tension the conduit held while minimizing construction cost. The relationships between mechanical and functional properties of xylem have been accumulated with increasing number of researches. In some studies, trade-off was found between hydraulic conductivity and mechanical support (Christensen-Dalsgaard *et al*., 2007; Jagels *et al*., 2003) whereas it was not the case for other (Awad *et al*., 2012; Pratt *et al*., 2007; Rosner *et al*., 2007; Rosner *et al*., 2008).

Wood density (*d*) is defined as the porosity, a ratio between areas occupies by cellulose wall over the total area (Hacke *et al*., 2001a). In some study, this trait is related to drought resistance of the species. High *d* was found with greater ability to withstand high xylem tension during drought stress (Ackerly, 2004; Hacke *et al*., 2000; Hacke *et al*., 2001a; Jacobsen *et al*., 2005). However, in some other studies (Cochard *et al*., 2007; Cochard *et al*., 2008; Lens *et al*., 2011); this trait is not associated with cavitation resistance. Furthermore, this relationship between *d* and vulnerability to cavitation which is firstly showed when comparing species does not hold within species (Awad *et al*., 2010; Awad *et al*., 2012; Mayr *et al*., 2003).

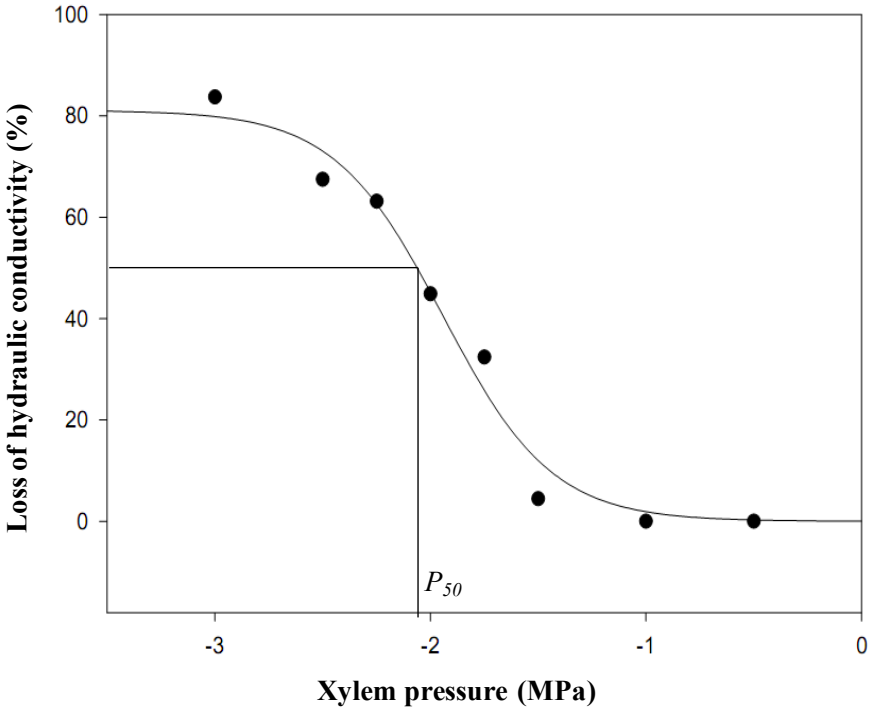


Figure 14. Xylem vulnerability curve to drought-induced cavitation.

The percentage loss of hydraulic conductivity (*PLC*) measured on sample is plotted as a function of xylem pressure (MPa). Data is fitted with exponential sigmoid function (Pammenter and Van der Willigen, 1998) and logistic line is obtained. The xylem pressure causing 50% loss of conductivity (P_{50}) which is indicated with the solid straight lines and the slope of vulnerability curve (*s*) are obtained from this fitting.

Considering the contrasted findings in the relationships between wood mechanical properties and the resistance to cavitation, the researchers might have to look further into the genetic control of these traits. It might allow us to assess the question on trade-off between the hydraulic and mechanical functions of wood and whether the mechanical traits could be used in the screening for drought resistance.

3. How to evaluate the drought-induced xylem cavitation?

Drought-induced cavitation is a serious problem for plant since it could lead to shortage in water supply then to organ or plant mortality. This is why it is important to measure the xylem vulnerability to cavitation of a species or a xylem sample. Xylem vulnerability to cavitation is generally quantified by relating degree of embolism as a function of water stress intensity (xylem pressure, *P*). Vulnerability curve (*VC*) is a representation of this relationship (Figure 14). Degree of embolism is generally appreciated by measuring its physiological consequence, that is to say the percentage loss of xylem hydraulic conductivity (*PLC*, %).

To construct vulnerability curve, the *PLC* is plotted versus xylem pressure and then data are fitted to an exponential sigmoidal function (Pammenter and Van der Willigen, 1998):

$$PLC = \frac{100}{1 + e^{\left(\frac{s}{25 \times (P - P_{50})}\right)}} \qquad (14)$$

where *s* is the slope of vulnerability curve and P_{50} is the pressure causing 50% loss of xylem conductivity (MPa). The P_{50} is commonly used as a parameter to compare the vulnerability to cavitation between samples (species, genotype, organs, and environmental effects).

From this equation, the pressure causing 12% and 88% loss of xylem conductivity (P_{12} and P_{88}) are calculated. They are considered as the air-entry-point in which the embolism begins (Sparks and Black, 1999) and the full embolism point which is the pressure before xylem becomes totally non-conductive (Domec and Gartner, 2001), respectively. Following equations indicate show the calculation for both parameters:

$$P_{12} = P_{50} + \frac{50}{s} \qquad (15)$$

$$P_{88} = P_{50} - \frac{50}{s} \qquad (16)$$

There is an ongoing debate on the reliability of the method used to measure the vulnerability to xylem cavitation (Choat *et al*., 2010; Cochard *et al*., 2010; Cochard *et al*., 2013; Delzon and Cochard, 2014; Ennajeh *et al*., 2011b; Jacobsen and Pratt, 2012; Sperry *et al*., 2012; Tobin *et al*., 2013; Torres-Ruiz *et al*., 2014), such that the frequency of cavitation events and the physiological importance of resistance to cavitation could be questioned. Because of the importance of the reliability of the measure in this PhD work, a specific section devoted to the different techniques with their pros and cons are developed, with an emphasis on the care to be taken for a reliable analysis.

3.1 *Cavitation and embolism detections*

The cavitation and embolism events are detected via various methods which, in general, could be grouped into 3 categories: the acoustic detection (Milburn, 1973; Tyree *et al.*, 1984), the anatomic detection (Lo and Salleo, 1991) and the hydraulic detection (Sperry *et al.*, 1988).

Acoustic detection

The acoustic detection is a first method that demonstrated that plants were living under the threat of cavitation. When a cavitation occurs, the large negative xylem pressure in the conduit is suddenly increases to the atmospheric pressure (close to 0 MPa). The releasing energy causes an acoustic emission; it occurs over a very broad spectrum of frequencies, from audible to ultrasonic (Milburn, 1973). This method is very limited due to the difficulty to determine whether the emission is produced by cavitation events or by other events (in case of the ultrasonic emission). Moreover, the method is more qualitative than quantitative and it is 'amnesic' in the sense that the number of cavitation events that have occurred before the onset of the recording is unknown (Cochard *et al.*, 2013).

The main advantage of the techniques based on the detection of acoustic emission is that they are non-destructive and non-invasive and could even be used under field conditions with the recent devices (Tyree and Sperry, 1989a). Another advantage of these techniques is a very high temporal resolution that could detect the time of occurrence of a cavitation event with great accuracy.

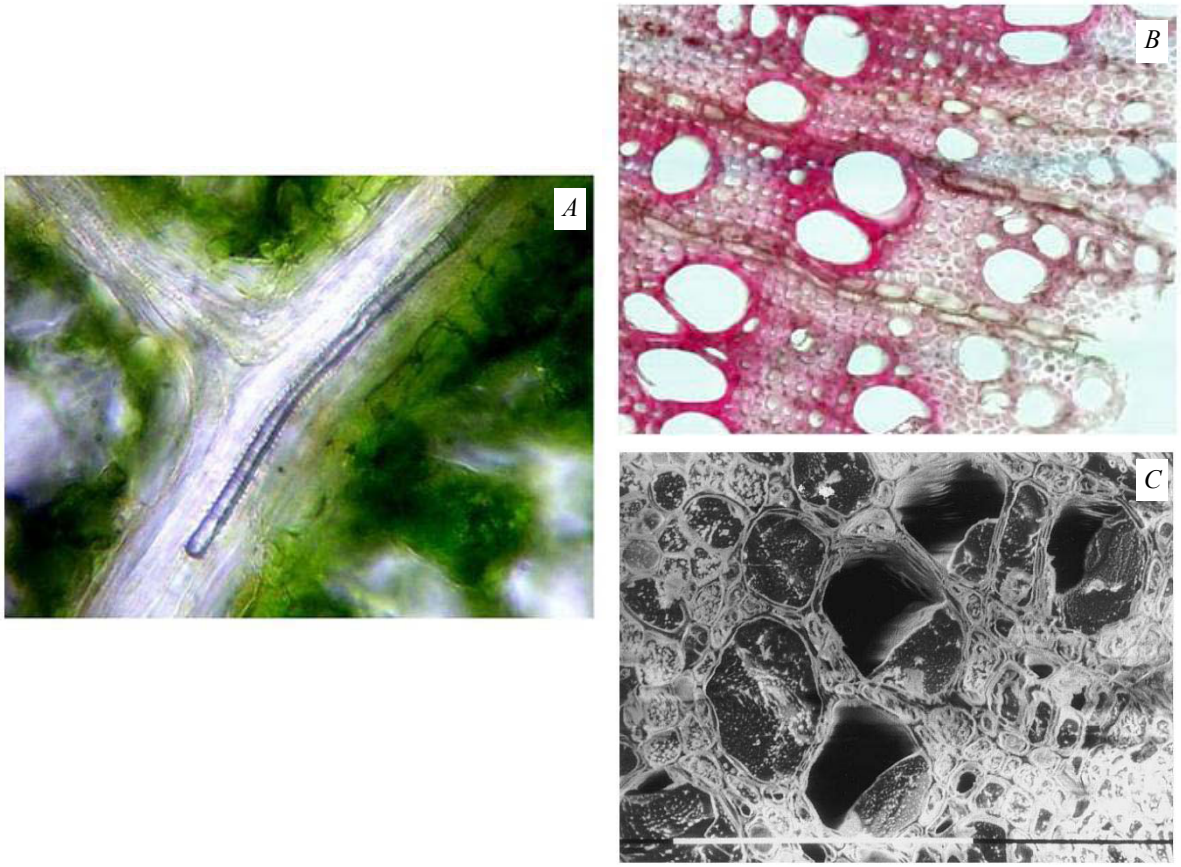


Figure 15. Observation of embolized vessels in plant.

A light micrograph shows an air-filled vein in *Juglans regia* leaf, (*A*). Cross-section of *Pistacia lentiscus* stem stained with basic fuchsin (*B*); the functional vessels were stained in red while non-functional ones remained unstained. Frozen *Juglans regia* petiole (*C*) observed with cryo-SEM. Vessels on the left side of the picture are entirely filled whereas on the right side, one-half-filled vessels are presented. Photographs are from Cochard and Delzon (2013), Vilagrosa *et al.* (2012), and Cochard *et al.* (2000), respectively.

Anatomic detection

The anatomical detection involved the direct observation of air bubbles presentation in the xylem lumens of thin axial wood sections. The air bubbles are observed by eye or under a light microscope (Figure 15) (Sperry and Tyree, 1988). The difficulty with this technique is to obtain reliable observations particularly during the preparation of thin wood section. The samples need to be prepared under water to prevent the entrance of air into the xylem conduits and the observation need to rapidly done because air bubbles dissolve with time (Lewis *et al.*, 1994). The very limited field of observation is another constraint of this method; it prevents a quantitative estimation of the total xylem dysfunction with this technique. However, in recent years, a number of more sophisticated technologies have been used for direct observations of xylem content such as using magnetic resonance imaging technology or a high-resolution X-ray computed microtomography system.

The indirect observation is a dye coloration method; it is a simple yet effective way of visualizing the presence of an air embolism in a xylem tissue. A dye like safranin, basic fuchsin, alcian blue, phloxine B is perfused into sample with low pressure (Lo and Salleo, 1991; Peguero-Pina *et al.*, 2011). The results of this observation are in agreement with more sophisticated techniques usually found (Cobb *et al.*, 2007; Hietz *et al.*, 2008). The used of cryo-scanning electron microscopy (cryo-SME) allows an accurate distinction between the water-filled and air-filled conduits (Cochard *et al.*, 2000; Cochard *et al.*, 2004).

Hydraulic detection

This principle is the most widely used. In our study, all embolism measurements were done using hydraulic techniques which consist to measure the loss of hydraulic conductance in xylem conduits due to embolism. The Xyl'EM (Bronkhorst, Montigny-les-Cormeilles, France) is a specific instrument devoted to this task. It measures the initial xylem conductance (K_i) of sample to be analyzed. Then, after removing the native embolism by flushing sample with pressurized solution, the maximum xylem conductance is measured (K_{max}). The percentage loss of xylem conductivity (PLC) is calculated as follows:

$$PLC = 100 \times (1 - \frac{K_i}{K_{\max}}) \quad (17)$$

The calculated *PLC* allows measuring a physiological embolism rate on the sample. In other word, this allows evaluating the physiological consequence (loss of conductance) of the native embolism.

3.2 *Methods to induce xylem cavitation*

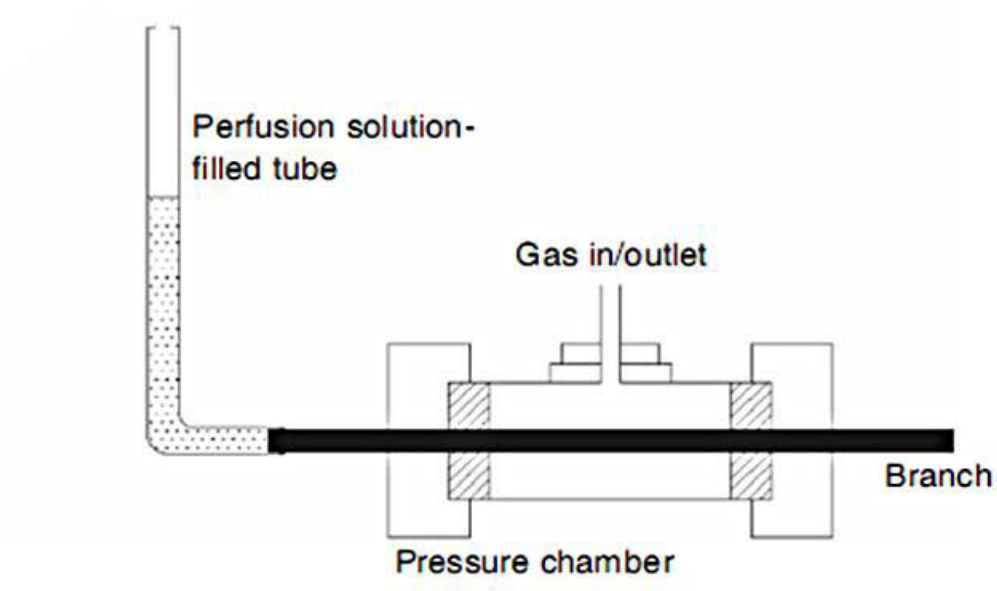
Several methods have been developed in order to explore the vulnerability to cavitation on the whole tree or on a segment of the tree. To construct a vulnerability curve, the dehydration level has to be controlled. The xylem pressure potential (*P*, MPa) is the key variable that is followed in relation with the embolism rate (Sperry *et al.*, 1988). Cavitation could be induced by various methods such as bench dehydration, air pressurization and centrifugation (Cochard *et al.*, 2013).

Bench dehydration

Bench-dry method (Sperry and Tyree, 1988) is considered a reference method because it is a natural way for inducing cavitation. Plant segment is left to dry out and afterward xylem pressure is measured using a pressure chamber on non-transpiring covered leaves or with stem psychrometers. The relevant pressure is the most negative pressure the plants have experienced during the drought treatment, usually during midday. This method requires a long time to induce cavitation in xylem. More, it is necessary to use a relatively large sample size (typically a leafy branch >1 m long) because very fast dehydration could induce a high heterogeneity of water stress in the branch and it should be avoided. The organ is cut from an intact plant and left to freely dehydrate in the air.

The study of Tyree *et al.* (1992) verified that VCs obtained from intact plants and cut branches are similar. However, the latter procedure is preferable as branch water status is better controlled.

To construct a vulnerability curve for evaluating the xylem vulnerability to cavitation, samples have to be exposed to several xylem pressures and their embolism rate measured. New samples which are exposed to increasing water stress are required. In general, it requires sizeable samples to construct a curve and as a consequence, the vulnerability curve obtained



Adopted from Ennajeh *et al.*, 2011

Figure 16. Scheme of the set up of the air-injection technique for measurement of xylem vulnerability to cavitation.

A customized pressure sleeve is applied to the center of branch segment where cavitation is induced by air pressurization. The distal end of branch segment is connected with a vertical solution-filled tube, solution flows from this tube through the segment and exits at the proximal end due to difference in water pressure. The solution is collected and weight to calculate flow rate. Modified from Ennajeh *et al.* (2011b).

is usually represented by a population of plants rather than a single plant or genotype. With the currently developed techniques: the air-injection technique using "pressure sleeve" and the centrifugal technique "Cavitron", vulnerability curve could be obtained from a single sample.

Air pressurization

According to air-seeding hypothesis, the rupture of water column occurs because of the pressure difference across an air–water meniscus located on xylem walls exceeds a critical value. Hence, decreasing *P* by dehydration under constant atmospheric pressure or increasing air pressure while maintaining the xylem pressure close to zero MPa should resulted in the same effect on cavitation. This method was firstly introduced by Crombie *et al.* (1985).

Various improvements have been carried out for this method, especially with the pressure sleeves (Cochard *et al.*, 1992; Salleo *et al.*, 1992). A pressure sleeve is put on the middle of sample segment to applied pressurized air (Figure 16). Cavitation is induced by air pressurization while the conductance is measured simultaneously by measuring the weight of solution flows through sample during a given time interval and at a given pressure. Afterward, air pressure in chamber is increased step by step to create higher embolism levels and the previous measuring steps are repeated until the complete *VC* is obtained.

The advantage of this technique is that cavitation induction could be manipulated with a high accuracy and in a short time. In addition, a whole *VC* could be constructed using only one sample within a few hours.

Centrifugation

Centrifugal force was used to exposed liquid water in Z-shaped Pyrex glass capillaries to large negative pressures (Briggs, 1950). The tensile strength at which cavitation occurred was measured; the tension that induced the breaking of the water column is referred as the cavitation pressure.

The principle of centrifugation technique is to spin a sample segment in a centrifuge to lower the xylem pressure in the middle part of the sample at a target pressure value. This pressure is computed according to the following equation:

$$P = -0.25\rho\omega^2 \times (R^2 + (R-r)^2) \qquad (18)$$

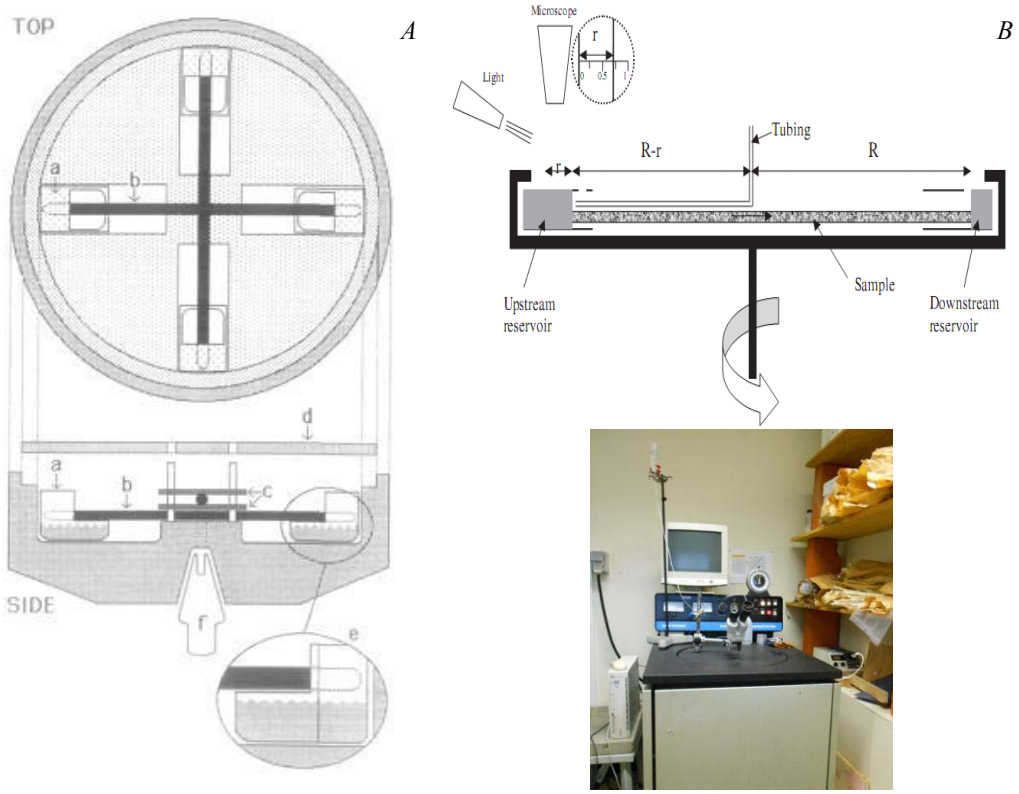


Figure 17. Centrifugation techniques for inducing and measuring xylem cavitation.

Drawing of a rotor and the set up for measurement of xylem vulnerability to cavitation using centrifugal technique from Alder *et al.* (1997); (*A*). The rotor was machined from a single piece of 7075 T6 aluminum. Slots in the main body retained plexiglass 'L' shaped reservoirs (a) are contained water. Segments (b) are held by thin aluminium plates (c) secured by nuts on the bolts that also hold the lid (d). A maximum of two segments could be spun at once, crossing in the middle and held by a sandwich of two retaining plates. Segment attachment plates and lid bolts are absent in the top view for clarity. The rotor is attached to the centrifuge shaft (f) by a bolt (not shown). During rotation, centrifugal force moves the water in the reservoirs to the position indicated by the dotted line in the insert (e), and immerses the segment ends. A drawing from Cochard *et al.* (2002b) and photograph of the set up for measurement of xylem vulnerability to cavitation using centrifugal technique 'Cavitron' (*B*). Sample is placed in the rotor with its center on the axis of a centrifuge, and with both ends immersed in solution contained in plastic reservoirs. The xylem pressure applied to the sample will thus depend both from the centrifugation velocity and the distance of the solution from the rotor axis (R). The solution levels are determined by the position of holes in reservoirs' wall such as there is a difference (r) in water level between both reservoirs. This difference in water levels creates a difference in water pressure, inducing a flow of the solution (*F*) through the sample from the upstream reservoir (with higher level of water) to downstream reservoir. In the downstream reservoir, the solution level is constant while, in the upstream reservoir, the solution level decreases from R-r to R over time proportionally to *F*. The value of r is determined optically during centrifugation by measuring the distance between the air–water meniscuses in the reservoirs. A tube is used to refill the system with more solution, hence allowing repetitive measurements.

where ρ is the density of water (1,000 kg m^{-3}), ω the angular velocity (rad s^{-1}), *R* the distance (m) from rotation axis to the downstream reservoir and *r* the difference of water levels between two water reservoirs.

Cavitation is induced in the xylem within seconds after sample is exposed to the spinning. This technique has the same advantage than the air injection method but without the inconvenience associated with the presence of pressurized air in the sample. It could be used with small herbaceous species that are not suitable for the air-injection technique such as Arabidopsis (Tixier *et al.*, 2013). The desired xylem pressure could also be obtained with a short time after the spinning started and the xylem pressure is known with considerable accuracy.

Alder *et al.* (1997) developed a method for the measurement of vulnerability to cavitation on plant segment with their cut ends immersed in water. It used a customized rotor which could accommodate a maximum of two segments to spin at once (Figure 17*A*). During the spinning, water in plexiglass 'L' shaped reservoir is moved to immerse segments' ends. For this method, the cut open xylem conduits at either end of the segment were open to the air. This allowed some of the water in them to evaporate and/or to be thrown out by the rotational motion. Hence, after a spinning, it was necessary to re-cut the segments underwater to remove the air-blocked ends before the measurement of K_h. As a consequence, a segment could only be used to one pressure. To complete the vulnerability curve, several segments were required (Pockman *et al.*, 1995). In addition, this technique was only applicable on segments that most of xylem conduits were rather short. Since after removed the blocked conduits at both ends, it is necessary to still had enough of a segment for K_h measurement.

The Cavitron (Cochard *et al.*, 2005) was later build accordingly (Figure 17*B*). A sample segment (usually around 0.3 m) is spun in a centrifuge while both ends of segment are always submerged under water in the reservoirs. The spinning lower xylem pressure in the middle part of the sample at a target pressure value and cavitation is induced. Xylem pressure (*P*) is, firstly, set to a reference pressure and the K_{max} is measured. Then after, xylem tension is increased by increasing the rotation velocity and new sample conductance (K_i) is measured. The process is repeated on the same sample with increment in xylem tension until *PLC* reaches more than 90%.

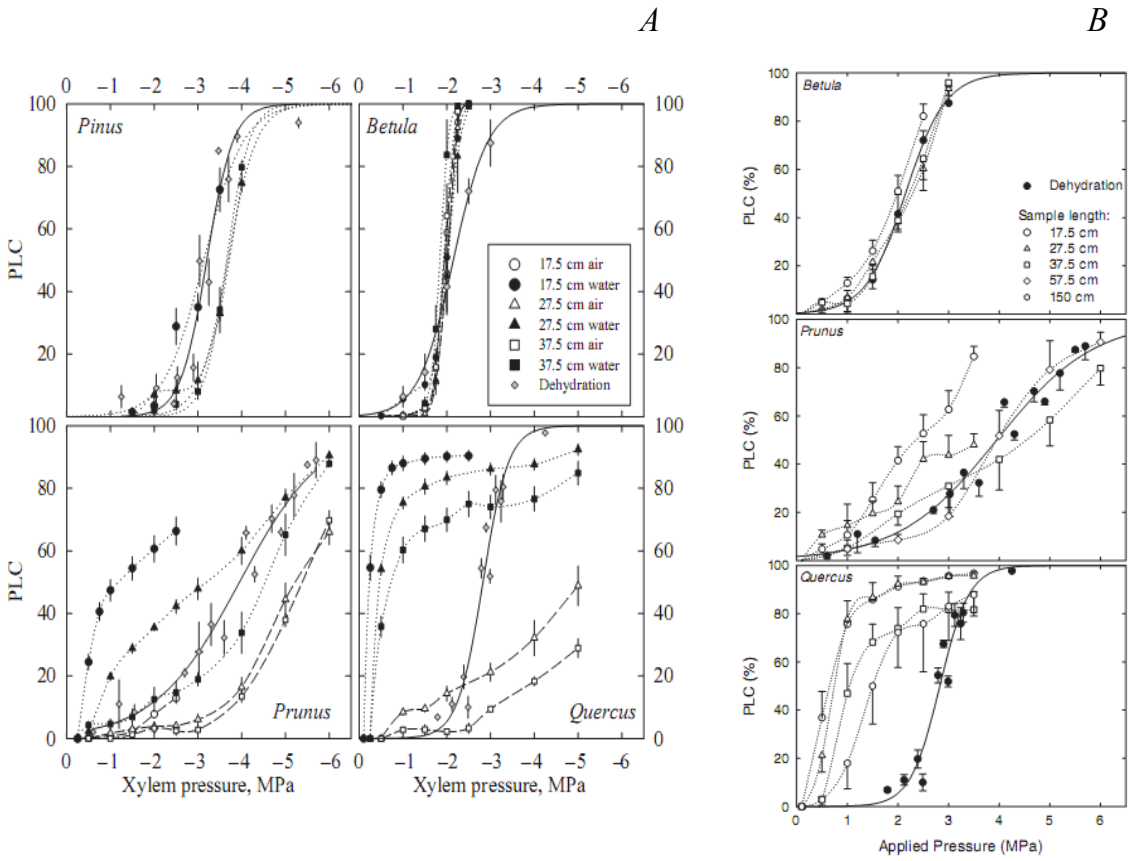


Figure 18. Effects of sample length on xylem VCs obtained with Cavitron and air-injection techniques on different segment lengths.

Different shapes of *VC* were obtained from Cavitron and air-injection techniques, demonstrating open vessel artifact. The vulnerability curves from Cavitron (*A*) are from Cochard *et al.* (2010); closed symbols represent samples of various lengths (different symbols) cut under water and centrifuged with the Cavitron technique and open symbols represent samples treated similarly but infiltrated with air at both ends before centrifugation. The grey symbols show vulnerability curves obtained with the reference bench dehydration technique. Error bars are *SE* (n = 4). Vulnerability curves in (*B*) are obtained from air-injection technique (white symbols) with a reference technique is shown with closed symbols (Ennajeh *et al*., 2011a). The length of the pressure sleeve was 3.5 cm and error bars are *SE* (n = 6).

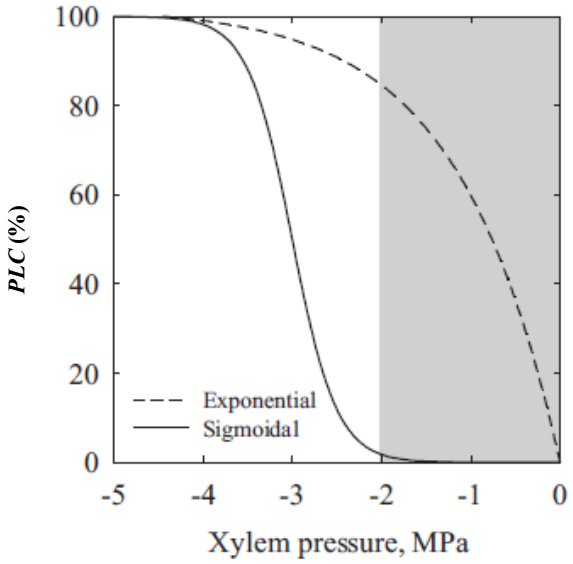
The improvement of the Cavitron from previous method is that it allows a measurement of sample conductance at the same time while sample is spinning which helps to reduce a considerable time of the experiment. Moreover, since the ends of segment are always immersed under water in the reservoirs, the measurement could be done on the same segment until vulnerability curve is completed.

3.3 *Vessel length and reliability of the techniques for measuring xylem vulnerability to cavitation*

Among the techniques to induce xylem cavitation, the dehydration or bench-dry technique is considered a reference way to induce cavitation. Indeed, dehydration of large branch segments is similar to what happened in nature. However, due to it requires a long period of time to let the segment to dry off, the use of faster techniques like Cavitron and air-injection are increased. Despite several advantages of Cavitron and air-injection technique, there is an ongoing debate on the reliability of these techniques especially when measuring xylem vulnerability to cavitation on long-vessel species (Choat *et al*., 2010; Cochard *et al*., 2010; Cochard *et al*., 2013; Delzon and Cochard, 2014; Ennajeh *et al*., 2011b; Jacobsen and Pratt, 2012; Sperry *et al*., 2012; Tobin *et al*., 2013; Torres-Ruiz *et al*., 2014).

Several studies have pointed out the need in considering vessel length of the species when measuring vulnerability to cavitation (Choat *et al*., 2010; Cochard *et al*., 2010; Delzon and Cochard, 2014; Ennajeh *et al*., 2011a; Torres-Ruiz *et al*., 2014). These studies have demonstrated that the xylem conduits were found far more vulnerable to cavitation when using Cavitron and air-injection methods on segments that have vessel length exceeding the segment length.

The first evidence of this concern comes from a study testing the effects of stem length on the vulnerability to cavitation using Cavitron (Cochard *et al.*, 2010): as the samples become shorter, they could become more vulnerable to cavitation when spun in the Cavitron (Figure 18*A*). The xylem vulnerability to cavitation measured on 4 species with different vessel lengths and different techniques is presented. Oak (*Quercus robur* L.) is a ring-porous species with very long vessels. Birch (*Betula pendula* Roth) is a diffuse-porous species with very short vessels. Peach (*Prunus persica* (L.) Batsch) has vessels of intermediate length and Scots pine (*Pinus sylvestris* L.) is a coniferous species with tracheids. Clear changes in shapes of *VC* from sigmoidal shape (call “s”-shape) to exponential-shape (called r-shape) are observed



Adopted from Cochard *et al*., 2013

Figure 19. Schematic representation of the two shapes of xylem vulnerability curve.

The sigmoidal curve, with s-shape, (solid line) is considered a normal vulnerability curve with a safety range of xylem pressure (gray zone) whereas the exponential curve, with r-shape, (dash line) would be an anomalous curve without safety pressure zone. This latter type of vulnerability curve is suspect to be a result of cut open vessel artifact using the centrifugal or air-injection techniques. From Cochard *et al.* (2013).

when the length of sample used approaching the maximum vessel length of species. The anomalous 'r'-shaped *VC*s are obtained on samples shorter than the maximum vessel length of species that means sample contains vessels that were open at both ends. This is consistent with the results obtained from centrifugation technique of other studies (Choat *et al.*, 2010; Torres-Ruiz *et al.*, 2014).

The effect of open vessel artifact was also found (Figure 18*B*) when measuring xylem vulnerability to cavitation with air-injection technique (Ennajeh *et al*., 2011a). The study shows the shift in vulnerability curve when measuring xylem vulnerability to cavitation with this technique on plant segments having open vessels at both ends. Several segment lengths were tested: 0.18, 0.28, 0.38, 0.57 and 1.50 m long with the air-injection techniques on three species having different maximum vessel length. The study shows that the *VC*s obtained on segments having no open vessels result in normal sigmoidal *VC*s which are concordant with the dehydration technique whereas the r-shape *VC*s were obtained when measuring the vulnerability to cavitation on segments having open vessels. Sigmoidal *VC*s were obtained from all segment lengths when measuring vulnerability to cavitation on *Betula pendula*, a species with approximately 0.16 m long maximum vessel length. The measurement on *Prunus persica* demonstrated a shift in *VC*s from s-type to r-type when the segment length was approached a species maximum vessel length (0.42 m). The *VC*s measured on *Quercus robur* were mostly r-type curves, except from the 1.5 m long segments where intermediate curve was obtained. For latter species, maximum vessel length was 1.34 m.

The sigmoid vulnerability curve (s-shape) is considered as a normal curve of the *VC*, with a safe range of xylem pressure for which embolism rate remains very low. This range of xylem pressure closely corresponds to a range of physiological xylem pressures usually found in natural habitats of the species. The exponential or "r-shape" curves are considered anomalous since they do not have the safety pressure range for the species to operate. As soon as the xylem tension is lowered, high embolism rate immediately occurs (Figure 19). Although some studies argued that the r-shape *VC* might not be anomalous (Jacobsen and Pratt, 2012; Sperry *et al.*, 2012; Tobin *et al.*, 2013) because their vulnerability to cavitation was similar compared to dehydration technique. This high vulnerability would indicate that high embolism rate would occur daily. It thus would imply a refilling process acting daily. This refilling process is questioned and it is called "miraculous" since the vessel refilling have to occur in a network of interconnected conduits under tension. In addition, it would require

energy for the refilling with positive pressure. These concerns on the refilling process argue for considering r-shape VC as anomalous. The increase in vulnerability to cavitation for short segment is believed to be a result of the vessel that was opened to center. While the segment is spinning on the Cavitron, air bubbles which formed during the segment preparation might enter into the open conduits. These bubbles would initiate cavitation when the xylem tension reached a threshold. In intact vessels (not open), the bubbles will be filtered through intervessel pit membrane and hence these intact conduits are considered safe from this artifact.

4. Variation of xylem vulnerability to cavitation

The P_{50} value could varies in different levels such as across the species, within the species or even between different organs on the same plant. These variations are considered as the result of the genetic control and/or the adjustment of the individual to environmental conditions, that is to say the phenotypic plasticity. In this following section, the variation of xylem vulnerability to cavitation is discussed in two aspects: the interspecific variation and the intraspecific variation. In addition, on the intraspecific level, the genetic variability and phenotypic plasticity are discussed.

4.1 *Interspecific variation*

Numerous studies have shown that the P_{50} is differed across the species (Choat *et al.*, 2012; Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003), with a large range of variation. The value could be as high as −0.04 MPa which was found on a liana or woody vine species, *Celastrus orbiculatus* (Tibbetts and Ewers, 2000) or very low as −14.1 MPa for conifers (Willson *et al.*, 2008). Several studies were carried out to investigate the xylem vulnerability to cavitation of herbaceous species such as in common bean (*Phaseolus vulgaris*), fireweed (*Chamerion angustifolium*), maize (*Zea ma*ys) and rice (*Oryza sativa*) with differences vulnerability to cavitation between these species (Cochard, 2002a; Holste *et al*., 2006; Maherali *et al*., 2009; Mencuccini and Comstock, 1999; Stiller *et al*., 2005). Focusing on broadleaved tree species, the vulnerability to cavitation found on angiosperms might rank from −0.09 MPa in *Albizia julibrissin* (Li *et al.*, 2008) up to −8.12 MPa in *Ceratonia siliqua* according to Cochard *et al.*, unpublished date in Choat *et al.* (2012). In gymnosperms, it could vary from −1.74 MPa in *Podocarpus latifolius* (Vander Willigen *et*

al., 2000) to −14.1 MPa in *Actinostrobus acuminatus* and *Juniperus pinchotii* (Choat *et al.*, 2012; Willson *et al.*, 2008).

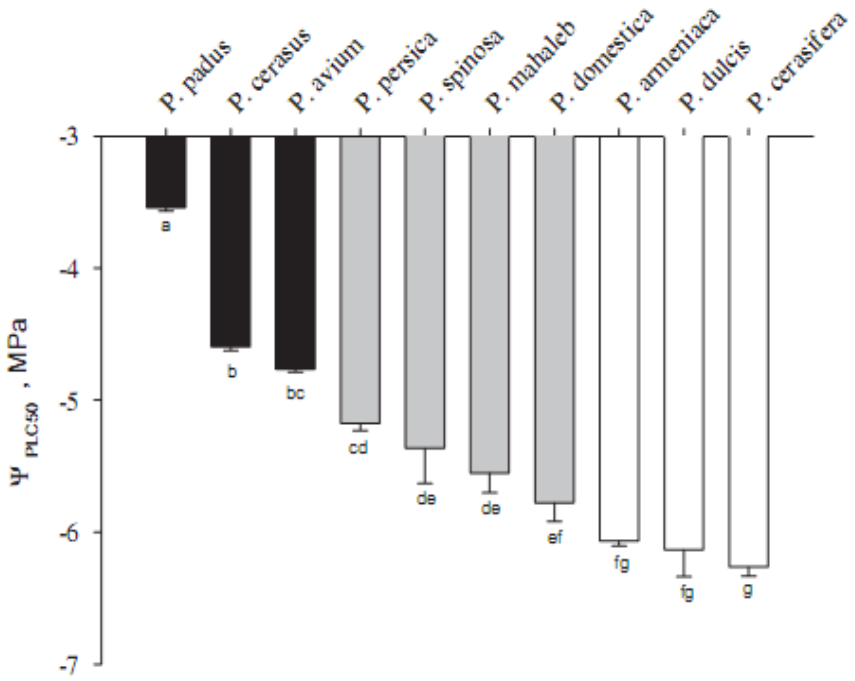


Figure 20. Vulnerability to cavitation (P_{50}) in 10 *Prunus* species.

The studied species were categorized into three groups according to the tension causing 50% loss of xylem conductivity (Ψ_{PLC50}, MPa): the vulnerable species with P_{50} above −5 MPa (*P. Padus, P. Cerasus* and *P. avium*), the resistance species with P_{50} below −6 MPa (*P. armeniaca, P. dulcis* and *P. cerasifera*) and the intermediate species (*P. persica, P. spinosa, P.mahaleb* and *P. domestica*). These three groups are presented with black, white and gray histograms, respectively. The values are mean (n = 4 – 5), error bars represent standard error (*SE*). Species not sharing a letter in common are significantly different at 95% confidence interval (Cochard *et al.*, 2008).

Variation in xylem vulnerability to cavitation was also found between species belonging to the same genus such as *Prunus* (Cochard *et al*., 2008) and *Acer* (Lens *et al*., 2011). Significant differences in xylem vulnerability to cavitation were found between ten *Prunus* species in a study of Cochard *et al.* (2008). These ten species comprised of four wild and six cultivated species: *P. padus*, *P. avium*, *P. spinosa*, *P. mahaleb*, *P. cerasifera*, *P. cerasus*, *P. persica*, *P. domestica*, *P. armeniaca*, and *P. dulcis*. They were selected to cover variation in water requirements, from hydrophilic to xerophilic types. The P_{50} of these ten species was ranked from −3.5 to −6.3 MPa (Figure 20) and correlated with species habitats supporting the relationship between cavitation resistance and drought resistance. In addition, the P_{50} was found to correlate with inter-vessel wall thickness of the species. In *Acer* (Lens *et al*., 2011), significant variation in P_{50} was found among seven species; the value was ranked from −1.26 to −3.33 MPa. The variation in this trait was found strongly correlated with intervessel pit membrane thickness, its porosity, pit chamber depth and mechanical strength parameters of xylem. A trade-off was also observed between hydraulic safety and efficiency in this study.

The variation in xylem vulnerability to cavitation across these species was found to correlated with their drought tolerance (Choat *et al.*, 2012; Cochard *et al.*, 2008; Delzon *et al.*, 2010; Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003). In a recent article of Choat *et al.* (2012), xylem vulnerability to cavitation was found related with mean annual rainfall (MAP) of the species (Figure 21). Decrease in vulnerability to cavitation was observed with the increasing MAP. This is the evidence showing that xylem vulnerability to cavitation is involved in the distribution and the resilience of species when facing drought stress.

4.2 *Intraspecific variation*

Despite the importance of variation of xylem vulnerability to cavitation on the intraspecific level which is crucial for the selection and screening for drought tolerance genotypes of agronomic species, fewer researches were conducted on this level. As previously mentioned that xylem vulnerability to cavitation varies on the interspecific level and relates to drought tolerance of the species, it might possible to find similar variation on the intraspecific level. In general, the intraspecific variation is smaller when compared with the interspecific variation. In the following section, intraspecific variation of xylem vulnerability to cavitation is discussed in term of the genetic variation and the phenotypic plasticity.

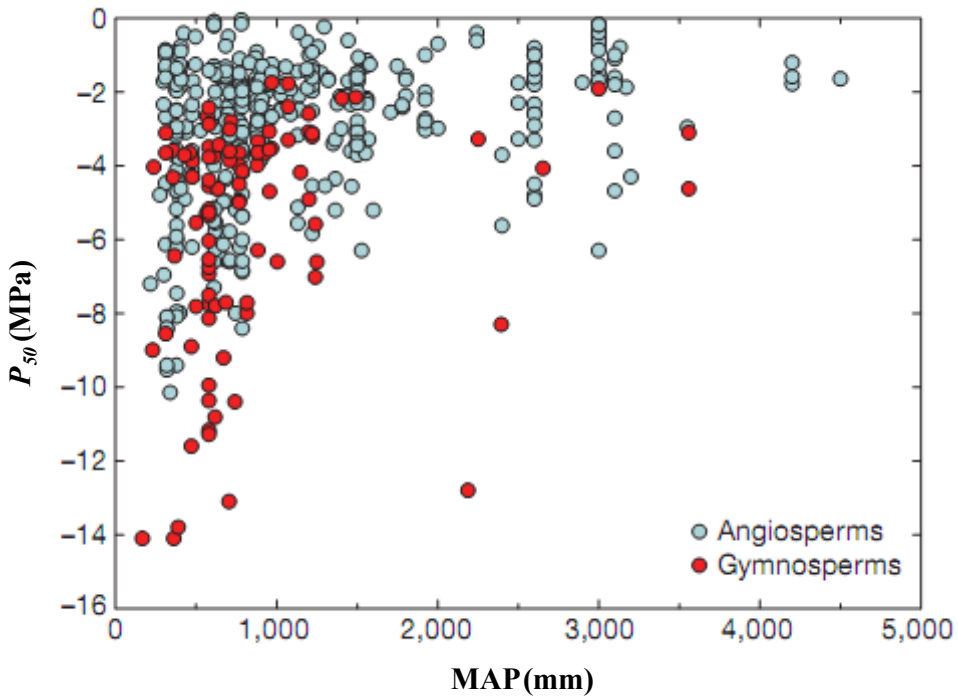


Figure 21. Vulnerability to cavitation (P_{50}) as a function of mean annual precipitation (MAP) of 480 species.

The P_{50} is xylem tension (MPa) which causes the 50% loss of conductivity whereas the MAP is mean precipitation (mm). Significant relationships were found between two traits ($P < 0.00001$) for both angiosperms and gymnosperms (Choat *et al.*, 2012).

Genetic variability

Xylem vulnerability to cavitation of the populations within a species could be varied under the genetic control similar to the variation found on the interspecific level. Several studies have shown this type of variation such as between clones of eucalyptus (Vander Willigen and Pammenter, 1998), between poplar and willow clones (Cochard *et al.*, 2007) and genotypes from an apple progeny (Lauri *et al.*, 2011).

In case of the eucalyptus, the vulnerability to cavitation significantly varied between four clones, however, the P_{50} ranked only from −1.31 to −1.77 MPa (Vander Willigen and Pammenter, 1998). The study of Cochard *et al.* (2007) aimed to investigate the variation of vulnerability to cavitation and its relation with yield in five poplars and four willow clones. It demonstrated significant differences in xylem vulnerability to cavitation among the poplar and willow clones with ranges of P_{50} being −1.9 to −2.2 and −1.6 to −1.9 MPa, respectively. Despite poor correlation in cavitation resistance and wood anatomical traits, the study has found a good negative correlation of cavitation resistance with aboveground biomass production. Although significant differences in vulnerability to cavitation were found in these studies, the range of P_{50} within species was much narrower than between species. Large variation in P_{50} was found among 90 genotypes from an apple progeny 'Starkrimson' x 'Granny Smith' (Lauri *et al*., 2011). The P_{50} was ranked from– 3.3 to −5.2 MPa with a good heritability (0.79). Large variation among these populations studied could explain by the fact that these genotypes were not under any selection pressure. The study also found that stem anatomy and morphology were not involved with hydraulic safety which is consistent with previous studies (Tyree and Zimmermann, 2002). In addition, there was no trade-off found between the hydraulic safety (P_{50} and s) and the hydraulic efficiency traits.

Even though there are numerous studies reported the genetic variability of xylem vulnerability to cavitation, this might be not the case for some populations. In addition, positive results, i.e. detection of a difference, are easier and often published than negative results, i.e. lack of variation. Less variation for cavitation resistance between populations than within populations was found on several studies, suggesting that this trait is canalized. In wild species, the action of natural selection could be categorized according to evolutionary biology by comparing neutral genetic differentiation between populations (FST) and genetic variation for quantitative traits (QST). Three possible outcomes are: QST > FST, QST = FST, QST < FST which indicate diversifying selection, genetic drift, uniform selection, respectively.

Low additive genetic variance between population and similar cavitation resistance among population appeared as the causes of canalization in cavitation resistance. This uniform selection and canalization in vulnerability to cavitation were first demonstrated on *Pinus* populations (Lamy *et al*., 2011; Sáenz-Romero *et al*., 2013), and applied on *Fagus sylvatica* (Wortemann *et al.*, 2011). The study explored vulnerability to cavitation between populations of *Pinus pinaster* in a common garden (Lamy *et al.*, 2011)and along the environmental gradients from warm and dry to cooler and wetter sites. There was no evidence of variation in vulnerability to cavitation along these populations; the averaged P_{50} was −3.73 ± 0.070 MPa. The lack of differences between populations for this trait was also found on another study on the populations of *Pinus hartwegii* along an altitudinal gradient, from 3,150 m to 3,650 m (Sáenz-Romero *et al.*, 2013). The averaged P_{50} of these populations was −3.42 ± 0.047 MPa. Another extensive study of cavitation resistance among 17 populations of *Fagus sylvatica* grown in provenance tests revealed a remarkably constant cavitation resistance across populations (Wortemann *et al*. 2011). The evidence from these studies suggested that genetic architecture could narrow trait variability to preserve functional phenotypes *in natura*.

Phenotypic plasticity

Phenotypic plasticity is defined as a modification of the phenotype which allows an organism or an organ to acclimate. It is a fundamental ability for organisms to cope with variation in environments and includes changes in morphological, physiological, behavioral, phonological, etc. The responses could be classified into two groups: a phenotypic variation which links to environmental changes and a variation under genotype-by-environment (G x E) interaction (Pigliucci, 2005). The G x E reaction is of interest for breeders in an attempt to develop plants that could adapt to large ranges of environmental conditions. Various studies shown the plasticity of vulnerability to cavitation in response to, for instant, water availability, light condition and nutrient availability.

Many Studies reported that plants grown under dryer environments tend to be less vulnerable to cavitation than ones grown at wetter areas. This trend is consisted with what previously found in genetic variability of xylem vulnerability to cavitation. It implies that plants might acclimate to dry condition and develop abilities to survive in this environment. A study on *Cordia alliodora* (Choat *et al.*, 2007) found significant variation in the P_{50} of trees grown at three tropical rainforests with different mean annual precipitation. In a study of Awad *et al.* (2010) clones of hybrid poplars (*Populus tremula x alba*) cultivated under contrasted water

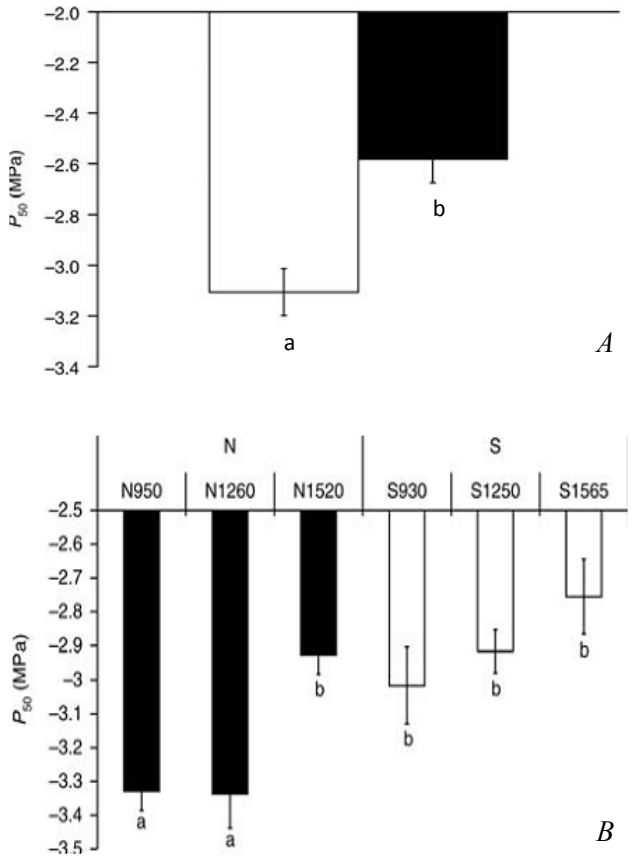


Figure 22. Pressure causing 50% loss of conductance (P_{50}) of *Fagus sylvatica* populations.

The P_{50} of samples from: (*A*) shaded (black histogram) and full light-exposed (white histogram), and (*B*) north- and south- facing mountainside (N and S, respectively) distributed along altitudinal gradients, for trees located on the N side at elevation 950, 1260 and 1520 m while the S side at elevation 930, 1250, 1565 m. Data are means (±SE) of 16 (*A*) and 6 (*B*) stems. From Herbette *et al*. (2010).

Table 1. Values of pressure causing 50% loss of xylem conductivity (P_{50}) in branches of seedlings of five species grown in 4, 16, 36 and 100% of full sunlight.

Irradiance	4%	16%	36%	100%
Betula pendula	−1.49 (0.023) a	−1.68 (0.026) b	−2.30 (0.089) c	−2.13 (0.043) c
Acer pseudoplatanus	−1.83 (0.13) a	−2.47 (0.038) b	−2.55 (0.038) b	−2.85 (0.039) c
Fagus sylvatica	−2.09 (0.019) a	−2.32 (0.020) b	−2.45 (0.021) c	−2.94 (0.041) d
Quercus robur	−2.25 (0.038) a	−2.46 (0.037) b	−2.63 (0.041) c	−2.91 (0.043) d
Quercus petraea	−2.32 (0.042) a	−2.56 (0.035) b	−2.69 (0.041) c	−2.94 (0.031) d

The P_{50} values are means of 12 replicates with standard errors in the brackets. Different letters within a species indicate significant differences at 95% confidence interval between different irradiance conditions. From Barigah *et al.* (2006).

regimes shown variation in vulnerability to cavitation. The P_{50} on well-watered trees was −1.82 MPa whereas on the trees grown under drought condition, P_{50} was lower (−2.45 MPa). There was a correlation between the cavitation resistance to the reduction in vessel diameter and increase in vessel wall thickness $(t/b)^2$. Phenotypic variation of xylem vulnerability to cavitation was also found between six *Pinus pinaster* populations from xeric and mesic provenances (Corcuera *et al*., 2011). Trees from xeric site appeared to be less vulnerable to drought-induced cavitation than trees from mesic site. Xylem vulnerability to cavitation was also found varied on the intraspecific level between *C. alliodora* trees grown at three sites that differed in mean annual precipitation (Choat *et al*., 2007). These results suggest that there is a correlation between the dryness of environmental condition and the resistance to cavitation.

There are also studies demonstrating the effect of light conditions on the vulnerability to cavitation. Since light is one of the factors determining plant-water relation, one could expect that the xylem vulnerability to cavitation might be modified by this factor. The acclimation to light therefore might determine tree survival especially for species grown in the understory level. The studies on *Fagus sylvatica* (Cochard *et al*., 1999; Herbette *et al*., 2010) have demonstrated that there is a light acclimation effect on vulnerability to cavitation. In the former study, it was conducted on both potted saplings and the mature trees grown in the forest. Samples which were exposed to light appear to be less vulnerable to cavitation than shaded samples: the means of P_{50} for one year old shoots for light-exposed and shaded were −3.22 and −2.43 MPa on the mature trees whereas they were −2.92 and −2.45 MPa on saplings, respectively. These results were confirmed by a study of Herbette *et al*. (2010) (Figure 22) on the same species where light-exposed stems (−3.1 MPa) are less vulnerable to cavitation than shade-exposed stems (−2.6 MPa). This study also found difference in P_{50} between populations along a latitudinal gradient, but not for an altitudinal gradient. In addition, variation also found between populations depending on the mountain side. The north-facing mountainside or the northern populations appeared less vulnerable than those grown on the southern-side or in the southern populations. Another study from Barigah *et al.* (2006) on seedlings of five species: *Acer pseudoplatanus*, *Betula pendula*, *Fagus sylvatica*, *Quercus robur* and *Quercus petraea* exposed to different irradiances also shown similar effect of light to vulnerability to cavitation (**Error! Reference source not found.**). Higher P_{50} was found for all species when levels of irradiance decreased.

Different nutrient conditions are also reported to affect the vulnerability to cavitation. The study of Plavcová and Hacke (2012) showed this effect on vulnerability to cavitation of a hybrid poplar (*Populus trichocarpa* × *Populus deltoids* clone H11-11). The saplings that grown with greater nitrogen fertilization (400 ml of 7.5 mM NH_4NO_3) were more vulnerable to cavitation compared to the control saplings (400 ml of 0.75 mM NH_4NO_3). The P_{50} of basal segment was −1.14 and −1.42 MPa for fertilized and control saplings respectively.

Within tree variation in xylem vulnerability to cavitation

Vulnerability to cavitation was also different between organs of the same plant (Hacke and Sauter, 1996; Rood *et al.*, 2000; Sangsing *et al.*, 2004; Tyree *et al.*, 1993). The study of Tyree *et al.* (1993) showed that petiole xylems of *Juglans regia* were more vulnerable to cavitation than stem xylems. This finding suggests segmentation in vulnerability to cavitation which allows plant discarding expendable organs in order to preserve more important organs from dehydration (Zimmermann, 1983). In contrast, some other studies have found that leaf xylem such as in the midribs and petioles are less vulnerable to cavitation when compared to branch xylem (Hacke and Sauter, 1996; Sangsing *et al*., 2004). Hacke and Sauter (1996) found that petioles of *Populus balsamifera*, a deciduous tree, were less vulnerable to cavitation compared to branches and its roots were the most vulnerable organ. A study on *Hevea brasiliensis* (Sangsing *et al.*, 2004) has also found that xylem in midrib are far less vulnerable to cavitation compared to petioles and branches with P_{50} ranking from −2.72 to −1.22 MPa.

Furthermore, the study of Cochard *et al*. (1997) demonstrated that even with the same type of organ but at the different positions on an individual tree, vulnerability to cavitation could differ. Leaf rachises from the lower canopy layer were found less vulnerable compared to the ones from upper layer; the P_{50} values of leaf rachises of *Fraxinus exculsior* from upper and lower layers were −2.5 and −3.5 MPa, respectively. This contrasted vulnerability to cavitation is related to xylem efficiency; the leaf rachises in upper layer had lower leaf specific conductance and smaller vessel sizes.

The vulnerability to cavitation is not only varied because of a genetic control but it is also under the influences of environmental condition and the interaction of both. These influences need to be clearly understood in order to use the vulnerability to cavitation as a criterion for drought tolerance selection in agronomic species. The relationship of this trait with drought tolerance within a species has to be firstly studied since the species might adopt different

mechanisms to cope with drought stress. Resistance to cavitation usually comes with trade-off such as high carbon cost for xylem structure to withstand the implosion. Trade-offs thus needs to be explored. Finally, given that drought resistance involves not only the cavitation resistance but also others mechanisms, this process have to be studied and the relationships with growth performance and yielding potential have to be studied.

Table 2. The species, distribution and important characteristics of *Juglans*.

Sections	Modern distribution		Species	Characteristics
Rhysocaryon (black walnuts)	America	North American	*Juglans californica* S. Wats., *Juglans hindsii* (Jeps.) Rehder, *Juglans major* (Torr. ex Sitsgr.) Heller, *Juglans microcarpa* Berl.,*Juglans mollis* Engelm. ex Hemsl., and *Juglans nigra* L.	Typically bear four-chambered nuts with thick nutshells and septa; thick, indehiscent and adherent husks; and are borne singly or in pairs.
		Central American	*Juglans olanchana* Standl. & L. O. Williams, *Juglans steyermarkii* Mann., and *Juglans guatemalensis* Mann.	
		South American	*Juglans australis* Griesb.,*Juglans boliviana* (C.DC.) Dode, *Juglans neotropica* Diels, and *Juglans venezuelensis* Mann.	
Cardiocaryon (Oriental butternuts)	East Asia		*Juglans ailantifolia* Carr., *Juglans cathayensis* Dode, and*Juglans mandshurica* Maxim.	Has two-chambered nuts with thick nutshells and septa, indehiscent and persistent husk, and are borne in long racemes of up to 20 nuts.
Trachycaryon (Butternuts)	Eastern North America		*Juglans cinerea* L	Bears two-chambered nuts with thick, rough shells featuring distinct sharp ridges and furrows on the surface, indehiscent and persistent husk, and are borne in clusters of 2–3 nuts on long stalks.
Juglans	Southeastern Europe to China and the Himalayas		*Juglans regia* L (Persian or English walnut) *Juglans sigillata* Dode (Iron walnut)	Bears four-chambered nuts generally singly or in pairs, occasionally three nuts, smooth, thin nutshells, and papery septa, and a dehiscent husk that separates easily from the nut. Have thick, rough-shelled nuts, and characteristic dark-colored kernels.

Classification of *Juglans* according to Louis-Albert Dode (Aradhya *et al*., 2007) which divided them into four sections: *Rhysocaryon*, *Cardiocaryon*, *Trachycaryon* and *Juglans*

V. Rationale for the choice of the species

In this study, we chose to work on three species: the walnut, the rubber and the apple trees. For walnut trees, the studied population included six commercialized Persian walnuts (*J. regia*) and six hybrid walnuts (*J. regia x J. nigra* NG 38), rubber tree are ten commercialized clones from Thailand and apple trees are five genotypes of a cross between 'Starkrimson' and 'Granny Smith'.

Their respective ecologies are different and the genetic structure of the populations is varied, which give more opportunities to explore the intra-species variation in xylem vulnerability to cavitation. In addition, for walnut and rubber trees, we used commercial cultivars and clones, respectively; they underwent the selections that aim for different agronomic objective: fruits and latex yielding the selection pressures on each species are different leading to different genetic structure. The walnut and rubber trees used in this study are commercial cultivars and clones respectively; they underwent the selections that aim for different agronomic objective: fruits and latex yielding, respectively. The apple genotypes were chosen base on their large variation in vulnerability to cavitation (P_{50}) and drought-induced stomatal closure ($FTSW_c$).

In the case of walnut and rubber trees, despite the potential to find differences in traits interested, the plant material we used was probably not the most appropriate. Our choice was carried out on plant material that was available at the period of our work. Therefore, the studies were carried out using as much as possible genetic diversity materials for each species. This is another reason for performing this work on three different species.

1. Walnut tree

The genus *Juglans* contains approximately 20 of deciduous tree species which are native from temperate to subtropical climates. Based on fruit morphology, wood anatomy, and foliage architecture, the *Juglans* could be divided into four sections according to Louis-Albert Dode (Table 2): *Rhysocaryon*, *Cardiocaryon*, *Trachycaryon* and *Juglans* (Aradhya *et al.*, 2007).

The *Juglans* have high value for their timber and edible nuts which are common in Europe, Asia and America. All species have 32 diploid chromosomes and many species are capable of hybridizing with each other (Germain, 2004). Among them, Persian walnut (*J. regia*) and eastern black walnut (*J. nigra*) have the most commercial values. The origin of Persian

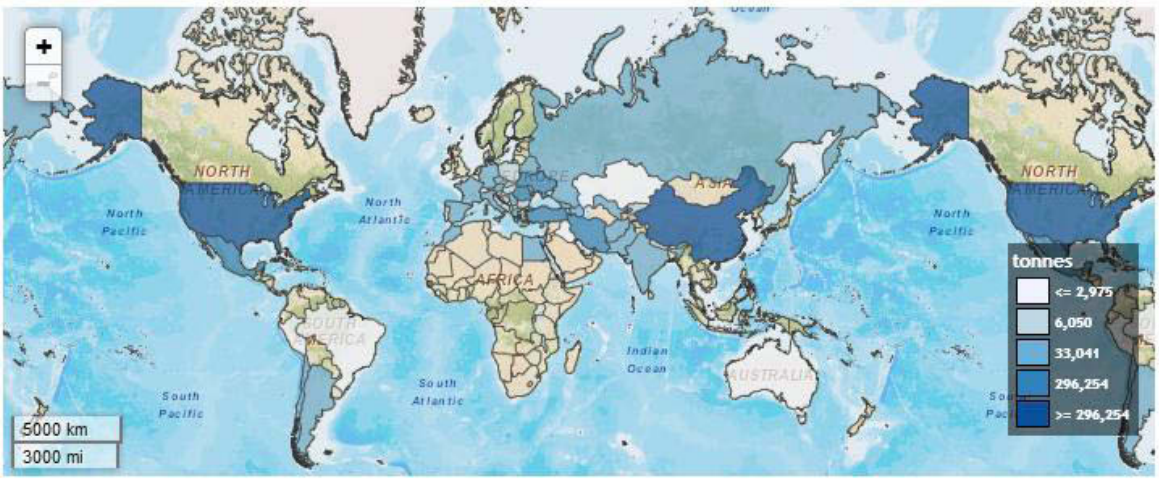


Figure 23. Walnut production by country.

World map show production of walnut with shells (tons) by country. Values are averaged from 1993 to 2013 (FAO, 2014).

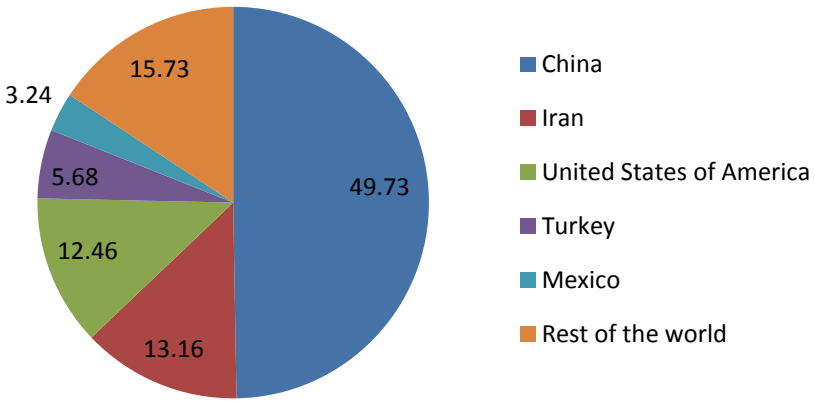


Figure 24. Origin of the walnut production in 2012.

Distribution of the production of walnut with shells (%) between the top 5 countries: China, Iran, the USA, Turkey and Mexico (FAO, 2014).

walnut or English walnut was in the mountain chains of central Asia which included the area of Turkey and Iran to China and Himalayas. Since its commercialization, it is largely cultivated throughout the world (Figure 23). Eastern black walnut is native to eastern USA and Canada. It is mostly cultivated for timber but its nuts also commercially used for confectioneries and ice cream. Despite some developed cultivars, most of them are still derived from wild or seedling trees. In Europe, this eastern black walnut is cultivated for timber and used as rootstock for orchard trees (Ramos, 1997). In the past decade, the production of walnut with shells was continuously increased. It rose from 1.42 million tons in 2002 to 3.42 million tons in 2012 according to FAO (2014). Approximately 50% of the production in 2012 came from China, followed by Iran, the USA, Turkey and Mexico while the remaining part of the production came from 49 other countries including Ukraine, India, Chile, France and Romania (Figure 24).

Persian walnut is self compatible; however, its genetic structure is rather heterozygous due to its dichogamy which encourages the allogamy of the species. Given the large variability in the area of distribution, phenology and some other physiological traits within *J. regia*, selection and breeding works were carried out in order to improve climate adaptation, early fruiting and high productivity, disease tolerance and high fruit and kernel quality of the species (Germain, 2004). Drought tolerance is one of the interesting traits for walnut breeding programs, since the important production sites include Mediterranean, semiarid and arid regions where more intense drought stress is likely to occur (Aletà *et al.*, 2009; Vahdati *et al.*, 2009). Currently, walnut production requires irrigation (Reid *et al.*, 2009) in order to ensure the optimal growth and productivity since walnuts require hefty amount of water (Fulton and Buchner, 2006).

Studies have been carried out in order to gain better knowledge on drought responses of walnuts to water stress (Gauthier and Jacobs, 2011) as an attempt to search for drought tolerance varieties for the coming future. The studies report that walnut tree adopts several drought avoidance strategies when facing water deficit. It develops deep root system (Pallardy and Rhoads, 1993) allowing tree to reach water at the lower soil depth and it has sensitive stomata to relative humidity which help in preserving water and preventing the occurrence of xylem embolism (Cochard *et al.*, 2002). In addition, walnut would display a segmentation of the xylem vulnerability to cavitation with petiole that was more sensitive to cavitation than branches (Tyree *et al.*, 1993). This would leave the leaves to shed in order to

Figure 25. Natural rubber production by country.

The world map shows the production of natural rubber (tonnes) by country. Values are averaged from 1993 to 2013 (FAO, 2014).

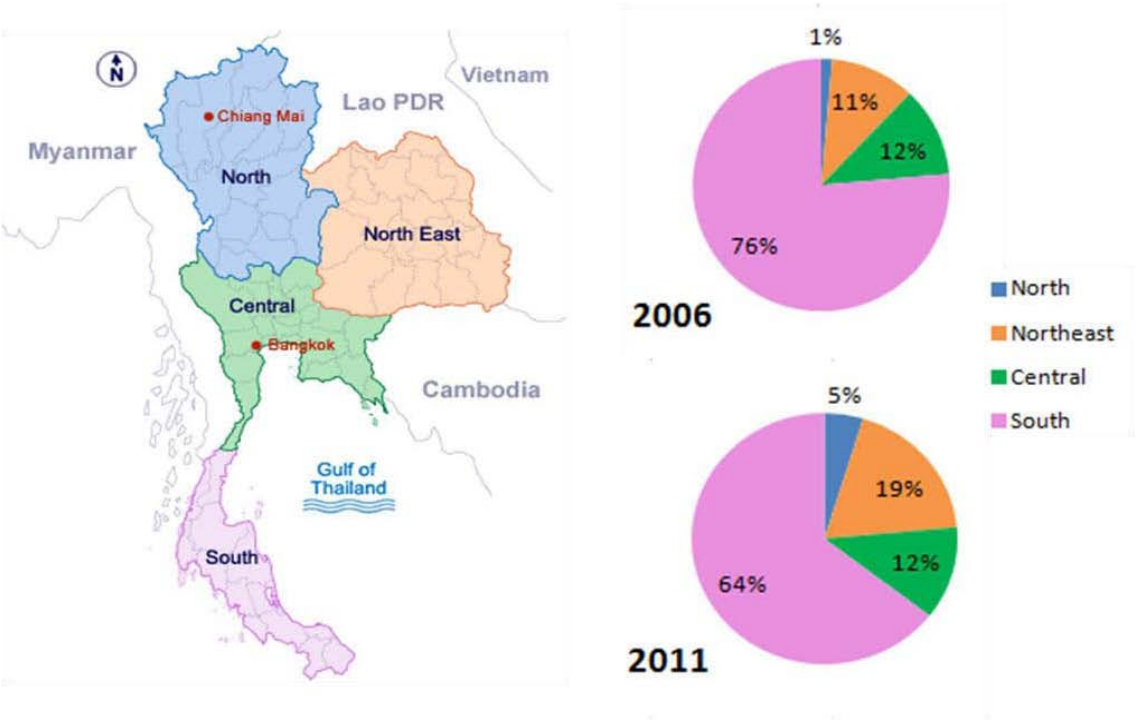


Figure 26. Distribution of the rubber plantations between regions of Thailand.

The distribution of rubber plantations (%) was shown between four parts of Thailand between for the years 2006 and 2011 (RRIT, 2011).

protect branches and stem from fully embolism. Studies demonstrated different drought responses among Persian walnuts (Aleta *et al*., 2009; Vahdati *et al*., 2009). There are differences in water use efficiency among the studied genotypes as well as differences in germination and growth rates when seedlings were grown under drought stress. These studies indicate a genetic potential for the screening of drought tolerance. Nevertheless, there was no insight on the genetic variability for the vulnerability to cavitation which is considered one of important drought tolerance traits.

2. Rubber tree

Para rubber tree, or rubber tree (*Hevea brasiliensis* Müll.Arg.), a native species from Amazonian basin, belongs to the family *Euphorbiaceae*. It favours warm and humid climates with average temperature ranks from 21 to 35°C and annual rainfall of 2,000 to 3,000 mm. In nature, the species distributes in the area between latitude of 15°N and 10°S (Duke, 1983). It is an important agronomic tree which is extensively cultivated for its latex (natural rubber) in various equatorial countries (Figure 25). It matures at the age of 7 to 10 years old, which latex tapping could be started.

The industrialization of this species started after 1839 with the discovering of vulcanization by Goodyear Tire and Rubber Company. At that time, latex was collected from the wild rubber trees grown in Amazonian forests. In 1876, rubber seeds were transferred from Brazil to the Royal Kew Botanical Garden in London by Sir Henry Wickham. The seedlings, descendants of these seeds, were later introduced to other countries in Southeast Asia such as Sri Lanka, Malaysia, Indonesia, India, Sarawak, and Thailand.

Several rubber seedlings were firstly introduced in the southern region of Thailand during 1899−1901 and afterward spread to the East part of country where climate conditions are favorable for growth of rubber tree. These 2 regions are considered the traditional production areas. During the beginning of natural rubber commercialization in Thailand, the cultivated rubber trees were the progeny of these seedlings that were firstly introduced into Thailand with rather low productivity. Rubber cultivation in Thailand was intensified due to the increasing demand for natural rubber in global market. In 1961, Thai government has launched a re-planting program for rubber (Rubber Estate Organization, 2014) in the traditional production areas, in order to replace the old native rubber trees with more suitable and more productive budded cultivars. Natural rubber production became very profitable

Table 3. Examples of the rubber clones recommended by Rubber Research Institute of Thailand (RRIT).

Class	Group	Clones for traditional production areas	Clones for marginal production areas
I	1	RRIT 251, RRIT 226, BPM 24, RRIM 600	RRIT 408, RRIT 251, RRIT 226, BPM 24, RRIM 600
	2	PB 235, PB 255, PB 260	RRII 118, PB 235
	3	Chachoengsao 50 (RRIT 402), AVROS 2037, BPM 1	Chachoengsao 50 (RRIT 402), AVROS 2037, BPM 1
II	1	RRIT 218, RRIT 250, RRIT 319, RRIT 405, RRIT 406, RRIT 410	RRIT 250, RRIT 319, RRIT 405, RRIT 406, RRIT 410, RRIT 411
	2	RRIT 312, RRIT 325, RRIT 403, RRIT 404, RRIT 407, RRIT 409	RRIT 312, RRIT 325, RRIT 403, RRIT 404, RRIT 407, RRIT 409
	3	RRIT 401, RRIT 414, RRIT415	RRIT 401, RRIT 414, RRIT 415
III		RRIT 3701, RRIT 3702, RRIT 3901, RRIT 3902, RRIT 3903, RRIT3904, RRIT 3905, RRIT 3906, RRIT 3907	RRIT 3710, RRIT 3702, RRIT 3901, RRIT 3902, RRIT 3903, RRIT3904, RRIT 3905, RRIT 3906, RRIT 3907

The rubber clones are recommended for the production in traditional and marginal areas of Thailand according to the RRIT (2012). Clones were classified into three groups according to their primary product: group 1 consists of latex production clones, group 2 includes clones that give both latex and wood, and group 3 includes clones for wood production. They are also classified according to their productivity and suitability for the production into three classes: *class I* consists of clones for large-scale planting with the production potential over 2,500 $kg.ha^{-1}.year^{-1}$ while *class II* and *class III* contains clones with good potentials of growth rate and productivity with estimated production potential range of 1,500−2,500 and less than 1,500 $kg.ha^{-1}.year^{-1}$, respectively.

activities and, in 1978, several plantation trails were set up at some provinces in the north-eastern region. It is considered a marginal area for natural rubber in term of water availability and soil properties. However, despite unfavorable conditions, growth and production in these marginal areas were found to be quite similar to the traditional production regions. The success of these production trails marks the beginning of northward expansion of rubber production in Thailand. Since then, the cultivation area was increased in these regions due to the abundances of lands and labors in the areas. According to RRIT (2011), the rubber plantation areas in the Northeast and North Thailand were increased by 12% from 2006 to 2011 (Figure 26).

Since 1991, Thailand has become the first producer and exporter of natural rubber in the world market with approximately 2.65 million tons of annual natural rubber production (averaged values from 1993 to 2013). Other important natural rubber producers consist of Indonesia, Malaysia, India and China which produced 0.53 to 2.05 million tons (FAO, 2014). Nowadays, natural rubber production (cultivation, transformation and trading) is one of the most important activities contributing to livelihood of around 10% of the Thai population.

Various rubber clones have been developed during the commercialization of the species. They are varied in vigor, growth, latex productivity, disease resistance, and tolerance to abiotic stress. However, only few clones were commercially used. In Thailand, most of rubber trees planted are the clone RRIM600; it occupied more than 80% of the total production areas (RRIT, 2011).

In general, the rubber clones recommended to the growers in Thailand (RRIT, 2012) could be categorized according to two factors: (*i*) the yielding potential and the suitability of the clones in large-scale production and (*ii*) the main product of the clones which could be the latex (*Group 1*) the latex and wood (*Group 2*) and the wood (*Group 3*). For latex yield, rubber clones are divided into three classes: *Class I* consists of clones for large-scale planting with the production potential over 2,500 $kg.ha^{-1}.year^{-1}$ while *Class II* and *Class III* contains clones with good potentials of growth rate and productivity with estimated production potential range of 1,500−2,500 and less than 1,500 $kg.ha^{-1}.year^{-1}$, respectively (Pratummintra, 2005). Considering lower production potential and some unknown information on clones' traits, the clones in *Class II* and *III* are recommended to grow in a limit area. Examples of recommended rubber clones for planting in Thailand are presented in Table 3 for both traditional and marginal production areas (RRIT, 2012).

Despite the economical profits, the expansion of rubber plantations northward into the marginal areas puts natural rubber production under a greater risk of drought stress. According to the Rubber Research Center of Thailand (RRIT, 2012), the suitable areas for rubber plantation should have at least 1,250 mm of annual rainfall with 120−150 raining days and without a dry period longer than 4 months. The average annual rainfall in these new plantation areas is just at the limit of the amount required by rubber tree (Thai Meteorological Department, 2012). Although the climate models has predicted the increasing in precipitations over Southeast Asia in the future, the increasing surface temperature and the longer dry period (IPCC, 2013) might amplify the risk of drought stress in these non-traditional production areas. Studies have shown that drought stress could affect growth (Chandrashekar *et al.*, 1998; Manmuen *et al.*, 1993) and latex yield (Raj *et al.*, 2005; Rao *et al.*, 1998; Wichichonchai and Manmeun, 1992) of rubber trees. Hence several practices such as weeding, mulching, ground cover cropping, branch pruning and, in case of severe drought, irrigation have been adopted for the cultivation (Office of the Rubber Replanting Aid Fund, 2003). Though these practices could prevent/decrease the damage from drought stress, they are very labor-intensive tasks and costly. Considering the intensive labor tasks for water management and the possibility of extreme drought events in the coming future (Chinvanno, 2011; IPCC, 2014), screening clones for drought tolerance become a subject of interest for rubber breeding programs.

The possibility of finding drought tolerant clones are highlighted by the differences in responses to water stress as demonstrated by various studies (Priyadarshan *et al*., 2005; Raj *et al*., 2005; Rao *et al*., 1990; Sangsing and Rattanawong, 2012).

3. Apple tree

The domesticated apple (*Malus domestica* Borkh.), originated from the central Asia, is one of the most cultivated temperate fruit species. It is widely distributed from the high latitudes in Europe, North America, and Asia to the high altitude equatorial zones. Most of apple cultivars are diploid (2n=2x=34) but many successful cultivars are triploids (3x=51). Triploid cultivars have larger fruit and are of value to the industry, but they need crossed pollination to have satisfactory yields, (Brown, 2003). Currently, there are over 10,000 named apples existing nowadays, yet not so many varieties are widely produced for international trade (Hampson, 2003). In commercialized apple production, vegetative propagation is used; the

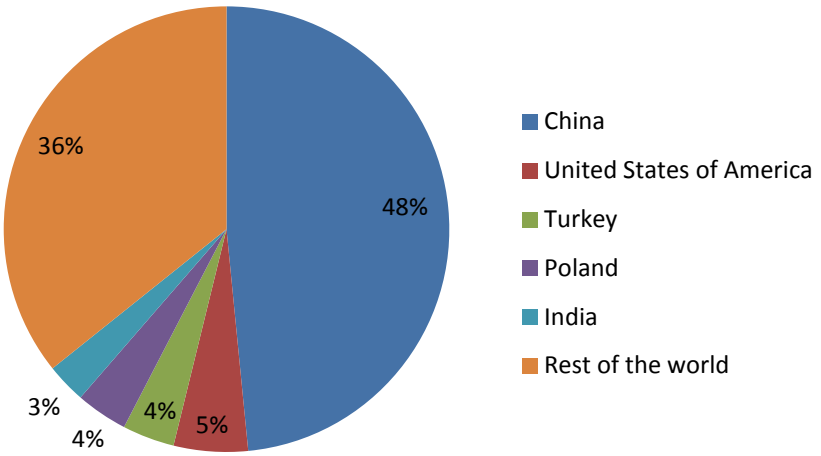


Figure 27. Origin of the apple production in 2012.

Distribution of the apple productions (%) between the top five countries: China, USA, Turkey, Poland and India (FAO, 2014).

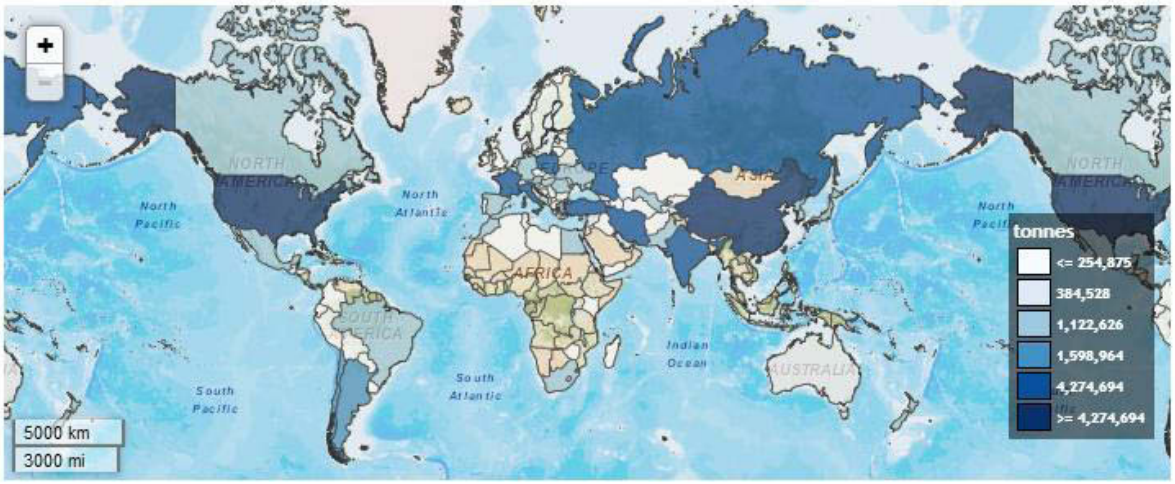


Figure 28. World distribution of the apple production.

World map shows the annual production of apples (tons) by country. The values represent mean obtained on from the period 2002−2012 (FAO, 2014).

selected cultivars are budded/ grafted on clonal rootstocks. While the scion varieties are selected for their fruit quality and quantity, the rootstocks are chosen for their vigor, persistent root system, disease resistance, and suitability to the environmental conditions of plantation.

In 2012, there were 76.38 million tons of apples produced over the world (Figure 27); the majority of the production (37.00 million tons) was from China, followed by the production from the USA (4.11 million tons), Turkey (2.89 million tons), Poland (2.88 million tons) and India (2.20 million tons), respectively. The rest of the production came from other smaller producers such as Italy, Iran, Chile, Russia and France (FAO, 2014).

Although most of the production is situated in the temperate zone, apple tree is also cultivated in semi-arid and arid regions as shown in Figure 28. In general, soil water content in apple orchard is maintained close to field capacity throughout the growing season because of a high water requirement of apple tree to maintain the optimal yield (Dragoni *et al.*, 2003; Green *et al.*, 2003). Hence, the production in drought-prone is heavily relies on irrigation system to ensure tree survival and productivity (Wertheim, 2003). Despite the availability of heat-tolerance varieties such as 'Granny Smith' and 'Fuji' and the development of irrigation techniques which ensure optimal irrigation doses (O'Rourke, 2003), searching for drought tolerance varieties is still a topic of interest for apple breeding programs since various apple production sites are under increasing risk of drought stress.

The studies on drought tolerance mechanisms were usually carried out on the root system (rootstock) and the aerial part (scion). On grafted tree, the degree of drought tolerance has to be considered as an interaction between rootstock and scion, since there are evidences of the influence of rootstocks (Landsberg and Jones, (1981). For apple tree, the rootstock influences water and nutrition uptakes, water transport, and canopy and tree size (Cohen and Naor, 2002; Li *et al.*, 2002). Various studies were carried out on ecophysiological traits related to drought response; however, most were focused on the rootstocks. Differences in drought tolerance were reported for apple rootstock, and they were studied for both root and leaf traits. The tolerant rootstocks tend to produce larger root dry matter which enhance hydraulic conductance in roots and therefore, allow the tree to be more tolerance to drought stress (Atkinson *et al.*, 1999). Few studies were carried out on the scion in relation to drought tolerance. The study of Angelocci and Valancogne (1993) shows good correlation between

apple leaf area and its water use. As an isohydric species, the apple tree closes its stomata early when facing soil water deficit. In the study of Regnard *et al*. (2009), they found that stomatal conductance of an apple progeny is strongly decreased with increased water vapor deficit (VPD). Massonnet *et al.* (2007) reported differences in water use strategies between the apple cultivars 'Braeburn' and 'Fuji', which result from different stomatal regulations. Atkinson *et al.* (2000) also showed differences in the stomatal response to water deficit on 9 different rootstocks.

Lauri *et al.* (2011) have found large variation for various anatomical and hydraulic traits in a progeny of 'Starkrimson' x 'Granny Smith', with high broad sense heritability (h^2_b). The heritability is the proportion of observed differences on a trait among individuals of population that is due to genetic differences. For the broad sense heritability, it reflects all the genetic contributions to a population's phenotypic variance including additive, dominant, and multigenic interactions, as well as maternal and paternal effects, where individuals are directly affected by the parent phenotype. Following are means of the whole progeny of some interesting traits with their first and seventh octiles values in the brackets: vessel density 65.8 $\times 10^{-5}$ μm^{-2} (55.0 $\times 10^{-5}$ to 75.7 $\times 10^{-5}$), hydraulic vessel diameter 18.9 μm (17.2 to 20.6), hydraulic conductivity 0.26 mmol s^{-1} MPa^{-1} m (0.14 to 0.35) and P_{50} −4.3 MPa (−5.2 to −3.3). The h^2_b values of these traits are: 0.70, 0.77, 0.77 and 0.79, respectively. Considering both the large diversity and the high heritability in cavitation resistance and hydraulic efficiency traits of the stem, they indicate a great potential for the screening of drought tolerance in this species.

OBJECTIVES OF THE STUDY

The literature shows that xylem vulnerability to cavitation in forest species could vary not only between species (Cochard *et al.*, 2008; Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003) but also within the populations of many forest tree species (Corcuera *et al.*, 2011; Herbette *et al.*, 2010; Maherali and DeLucia, 2000; Martínez-Vilalta *et al.*, 2002). Moreover, at the interspecific level, relationship was found between the vulnerability to cavitation and drought tolerance (Choat *et al.*, 2012; Cochard *et al.*, 2008; Delzon *et al.*, 2010; Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003). These findings suggested xylem vulnerability might be used as a criterion for drought tolerance screening. The possibility of using this trait was highlighted by the current reliable and fast analytical techniques (Cochard *et al.*, 2007; Cochard *et al.*, 2008).

To use xylem vulnerability to cavitation as a drought tolerance screening criterion, firstly, the investigation on the variation of this trait within the interested population and its heritability had to be carried out. Secondly, it was necessary to clearly understand the sources of variation of xylem vulnerability to cavitation since this trait was not only under genetic control but also under the influences of environmental condition and the interaction of both. Thirdly, the relationship between xylem vulnerability to cavitation and drought tolerance had to verified on the intraspecific level. Given that drought tolerance involves not only the cavitation resistance but also others mechanisms, it was important to also understand these mechanism. Finally, the relationship of this trait with growth and yield performances had to be studied. To date, the knowledge of these aspects on agronomic species is still scarce.

This study was carried out with the anticipation to improve the insights on the variation of vulnerability to cavitation, especially for agronomic tree species and the possibility of using this trait in drought tolerance screening. Hence the main objectives of this study were:

(i) to investigate the variability of xylem vulnerability to cavitation on tree species of agronomic interest, and

(ii) to test, if any, the relationship between xylem vulnerability to cavitation and the tolerance to a severe drought.

Three species: walnut tree, rubber tree and apple tree were chosen for this study. The walnut trees used in this study were accessions comprised of six of commercialized Persian walnuts (*J. regia*) and six hybrid walnuts (*J. regia* x *J. nigra* NG 38). The rubber trees included ten

commercialized clones used in Thailand and the apple trees were five genotypes from a cross between 'Starkrimson' and 'Granny Smith'. These three species are threatened by drought, have high economical values and vastly cultivated in a large range over the world.

We chose these three tree species because of the opportunity to explore the variability of vulnerability to cavitation in plant materials with different features, especially the differences on their sensitivity to cavitation and their genetic structure. The three species originated from different climatic regions and subsequently, they were differed in the sensitivity to drought stress. Walnut and apple trees originated from dryer regions of central Asia, they thus could be cultivated in Mediterranean, semi-arid and arid areas. Rubber tree came from tropical region of Brazil and it occupied mostly within warm and wet conditions of tropical region. Populations of the chosen species were expected to have enough genetic variability which allowed the variation of interested traits. For the populations of walnut and rubber trees, differences in drought responses, geological distribution, growth and yield were used to assume the genetic variability of these respective populations. These different features suggested the possibility of variation in vulnerability to cavitation. The choice of apple trees relied on the finding from Lauri *et al*. (2011) which large variations in P_{50} and desiccation avoidance behavior were found among the progeny. Five genotypes with contrasted drought tolerance strategies were selected for this study.

Another reason for choosing them is that we had access to these plant materials thanks to collaboration with Dr. Pierre-Eric LAURI (INRA Montpellier) and Pr. Jean-Luc REGNARD (Montpellier SupAgro, Montpellier) for apple tree and thanks to collaboration with researchers from the Rubber Research Institute of Thailand (RRIT) including Miss Ratchanee RATANAWONG for rubber tree, while genotypes of walnut tree were planted in our site (INRA Crouël).

The principal method for measuring xylem vulnerability to cavitation in this study was the Cavitron, a centrifugation technique that allows a measurement of water flow through sample while it was exposing to different level of stress (different velocity of the spinning). For the experiment on walnut and rubber trees, we also tested the protocols, the techniques and sample conditions on the vulnerability to cavitation of the species. These sub-experiments

aimed to find the most suitable method to carry out the measurement of xylem vulnerability to cavitation with the Cavitron and to compare the result with air-injection technique.

Drought tolerance is a complex process and might involve many mechanisms; therefore, it is important to understand whole plant response to drought stress to be able to identify any pertinent trait for the selection. In the experiment on rubber and apple trees, tree response such as stomatal regulation, embolism rate, leaf water potential and growth were followed through the progression of drought stress. The sensitivity to drought stress of the studied populations was monitored via changes in growth and embolism rate during the experiment. The following section describes in detail the aims of the experiment for each tree species.

Walnut tree

The investigated walnut trees were Persian (*Juglas regia*) and hybrid (*J. regia* x *J. nigra* NG 38) walnuts. The former are fruit-oriented while the latter are wood-oriented. Aim of this study was to evaluate the genetic variation of vulnerability to cavitation in these *Juglans* accessions. By using these two species, it not only allowed us to study the variation of xylem vulnerability to cavitation within the species but also permitted a comparison of variability between two species. In addition, to improve the accuracy of the Cavitron technique to assess xylem vulnerability to cavitation with this species, analytical methods were tested. Then after, xylem vulnerability to cavitation in six Persian and six hybrid walnuts was measured using the most suitable protocol. Structural traits in relation with xylem vulnerability to cavitation also explored for the six Persian walnuts.

Rubber tree

The studied rubber trees (*Hevea brasiliensis*) include ten commercialized clones from Thailand: BPM 24, PB 217, PB 235, PB 260, PB 5/51, RRII 105, RRII 118, RRIM 600, RRIT 251 and RRIT 408. All of them derived from a single population called ‘Wickham’s population’. They were Brazilian rubber seeds, brought back to Kew Botanical Garden by Sir Henry Wickham in 1879. Since this introduction of rubber trees, commercialized clones were developed in different research centers located in different countries over the world.

Two main objectives in this study were to investigate: the genetic variability of xylem vulnerability to cavitation between these clones and the response to a severe drought.

Before the investigation of vulnerability to cavitation, the feasibility of techniques for measuring this trait was explored, given that the suitability of centrifugation "Cavitron" and air-injection techniques had not been tested with this species. Different protocols for the measurement and different sample conditions were studied to improve the protocol of measurement. Afterward, genetic variability of xylem vulnerability to cavitation was carried out on the ten rubber clones. In addition to the genetic variability in xylem vulnerability to cavitation, the plasticity of this trait was also studied. Samples of two rubber clones: RRIM 600 and RRIT 251 from trees grown at three different locations were measured. These locations differ in mean annual rainfall and thus in the water regime of their respective plantation.

Lastly, the experiments on plant responses to drought stress were carried out on one-year-old trees of these clones. It aimed to compare clones for their drought response for young individual of this species and to help in identifying pertinent traits for drought tolerance screening.

Apple tree

The apple genotypes used in this study belong to a progeny from a cross between 'Starkrimson' and 'Granny Smith'. Among the 122 individuals of the progeny, five of them: B0023, B0057, B0070, B0097 and B0111 were chosen. They were differed in vulnerability to cavitation (P_{50}) and drought-induced stomatal closure ($FTSW_c$). Cavitation resistance was focused as a desiccation tolerance trait while stomatal regulation was chosen as a trait representing avoidance behavior in this study. Genotype B0023 was characterized as a cavitation sensitive and water spending genotype (P_{50} = −2.52 MPa, $FTSW_c$ = 72%), B0057 a tolerant and water saving genotype (P_{50} = −5.84 MPa, $FTSW_c$ = 82%), B0070 a tolerant and water spending genotype (P_{50} = −6.77 MPa, $FTSW_c$ = 46%), B0097 a cavitation sensitive and water saving genotype (P_{50} = −3.66 MPa, $FTSW_c$ = 93%) and B0111 a cavitation sensitive and water spending (P_{50} = −3.70 MPa, $FTSW_c$ = 49%).

Objective of this experiment was to investigate their response to a severe drought in relation with both traits (P_{50} and $FTSW_c$). The experiment on plant response to a severe drought was conducted during two growing seasons in 2012 and repeated in 2013. Between these two experimental years, apple trees were grown with different irrigation regimes: in 2012, trees were grown at full field capacity whereas in 2013, trees were grown under mild drought

stress. We expected that by lower the available water during growth period will enhance different responses to drought stress from these genotypes.

METHODOLOGY

I. Plant materials and experimental designs

1. Walnut trees: the investigation of xylem vulnerability to cavitation
2. Rubber trees
    - *2.1 The investigation of xylem vulnerability to cavitation*
        - Feasibility of the techniques and effects of sample conditions on xylem vulnerability to cavitation
        - Variation in xylem vulnerability to cavitation
    - *2.2 The responses to drought stress*
3. Apple trees
    - *3.1 The investigation of xylem vulnerability to cavitation*
    - *3.2 The responses to drought stress*

II. Methods

1. Measurements of xylem vulnerability to cavitation
    - *1.1 Evaluation of the vessel length*
    - *1.2 Cavitron*
    - *1.3 Air-injection*
2. Measurements of hydric traits
    - *2.1 Stomatal conductance*
    - *2.2 Leaf and stem water potential*
    - *2.3 Stomatal response to water potential*
    - *2.4 Xylem embolism*
3. Measurement of soil water content
4. Measurement of leaf and growth traits
    - *4.1 Leaf area and leaf mass per area*
    - *4.2 Tree growth*
5. Measurements of wood traits
    - *5.1 Wood density*
    - *5.2 Wood anatomical traits*

III. Statistical analysis

Table 4. Persian walnut cultivars used in the study with their origins and parents.

Cultivar	Origin	Parentage
Chandler	The USA	Pedro x UC56-224
Fernette	France	Franquette x Lara
Fernor	France	Franquette x Lara
Franquette	France	Unknown
Lara	France	Tulare x Chico
Serr	The USA	Payne x PI 159568

The origins and parentages of six Persian walnut cultivars: Chandlers (Ch), Fernettes (Ft), Fernors (Fo), Franquettes (Fq), Laras (La), and Serrs (Se) are listed.

This chapter is dealing with the vegetative materials, the experimental designs and methods for conducting the interested traits in this thesis. Firstly, plant materials of the three species: walnut, rubber and apple trees and experimental designs or the respective species are presented. After that, the methods used in the study are listed.

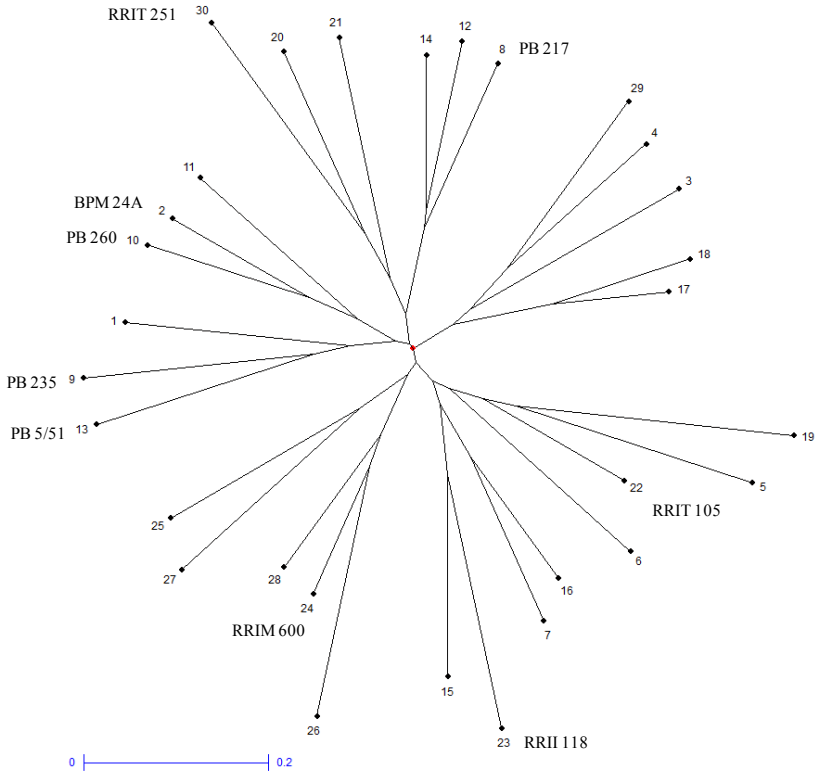
I. Plant materials and experimental designs

1. Walnut trees: the investigation of xylem vulnerability to cavitation

The investigation on xylem vulnerability to cavitation using Cavitron technique was conducted on two species of *Juglans*: Persian walnuts (*Juglans regia* L.) and hybrid walnuts (*J. regia* x *J. nigra*). Branch samples were harvested from 15 to 20 years old trees grown in orchard at INRA site Crouël, Clermont-Ferrand in south-central France (45°46′27″N, 3°8′36″E; altitude 338 m).

The Persian walnut trees were comprised of 48 individuals from six cultivars: Chandlers (Ch, 4 individuals), Fernettes (Ft, 3), Fernors (Fo, 4), Franquettes (Fq, 17), Laras (La, 15), and Serrs (Se, 4). They are important commercial cultivars for nut productions in France and the USA with different origins and parentages (McGranahan and Gale, 1994) (Table 4). On October 2011, 17 branches were randomly harvested from some of the 48 Persian walnut trees for a preliminary test with the Cavitron. The branches were mature, current year, at the least 0.45 m long and harvested from the southern side of the canopy (fully exposed to sunlight). After they were harvested, branches were immediately defoliated, wrapped with moist paper then placed in plastic bag to avoid dehydration and stored in cold storage (4°C) for maximum three days before the analysis was completed. These branches were used to test the suitable length of sample (0.28 and 0.38 m) and the direction of water flow through sample while spinning on the Cavitron (sense and antisense flows). The results from these measurements were used as a protocol for further investigation of xylem vulnerability to cavitation.

Three to six branches were collected from each individual Persian walnut tree on November 2011 for the investigation of genetic variability of vulnerability to cavitation. The sampling was done following similar harvesting and preparing protocol previously mentioned. Twenty branches were randomly chosen from these sampled populations for native embolism



Number	Clone
1	AVROS2037
2	BPM24A
3	BPM24B
4	GT1
5	Harbel1
6	Nab17
7	OY1
8	PB217
9	PB235
10	PB260
11	PB28/59
12	PB280
13	PB5/51
14	PB86
15	PR107
16	PR255
17	PR261
18	Reyan88-13
19	RRIC100
20	RRIC103
21	RRIC121
22	RRII105
23	RRII118
24	RRIM600
25	RRIM612
26	RRIM703
27	RRIV1
28	TJIR1
29	Yunyan77-2
30	RRIT251

Figure 29. Genetic diversity of 30 Wickham rubber clones based on seven microsatellite markers (Darwin, Cirad software for genetic diversity assessment).

Nine out of ten clones used in this study are presented in the diagram except clone RRIT 408 which has not been analyzed for its genetic. Figure is provided by André Clément-Demange, UMR AGAP, CIRAD, Montpellier.

measurement while the rest were stored in cold storage (4°C) up to 30 days before the analysis of their xylem vulnerability to cavitation. In addition, four leaves from each harvested branch were collected for the analysis of leaf mass per area (*LMA*).

To enhance the genetic diversity and to increase the possibility to explore the variation in xylem vulnerability to cavitation, further investigation on xylem vulnerability to cavitation was carried out using six wood-oriented hybrid walnuts (*J.regia x J. nigra* cv NG 38): F1, F2, H3, J3, N1 and N3. For each hybrid, 0.60 − 1.00 m long, current year branch (n = 8 – 10) were collected on November 2013. Native embolism was immediately measured after branches were harvested on 12 representative branches. Xylem vulnerability to cavitation was measured afterward on four samples for each hybrid.

The vulnerability curve (*VC*) of these samples was constructed by plotting *PLC* versus xylem tension. For each curve, the raw data were fitted using the sigmoid function (equation *14*). The features of xylem vulnerability to cavitation (P_{12}, P_{50} and P_{88}) were then calculated. The samples of Persian walnut branches were kept in a freezer and later, some of them were used for wood density measurement and observation of wood anatomy.

2. Rubber trees

The study on this species was carried out on ten commercial rubber clones from Thailand: BPM 24, PB 217, PB 235, PB 260, PB 5/51, RRII 105, RRII 118, RRIM 600, RRIT 251 and RRIT 408. These clones were derived from the rubber seeds of Wickham rubber populations which transferred from Brazil to the Royal Botanical Garden Kew, London in 1876 by Sir Henry Wickham. The chosen clones were considered to vary in their genetic structure. Nine out of the ten clones were analyzed for their genetic based on seven microsatellite markers (Clément-Demange, personal communication, June 7, 2013); they were well-distributed over 30 Wickham rubber clones (Figure 29). The clone RRIT 408 (PB 5/51 x RRIC 101), a recently selected clone for the cultivation in marginal areas of Thailand, was not included in this analysis. This clone was reported to have high growth rate and high latex yield (RRIT, 2012).

The variability of xylem vulnerability to cavitation and the clonal response to drought stress were the two main investigations for this species.

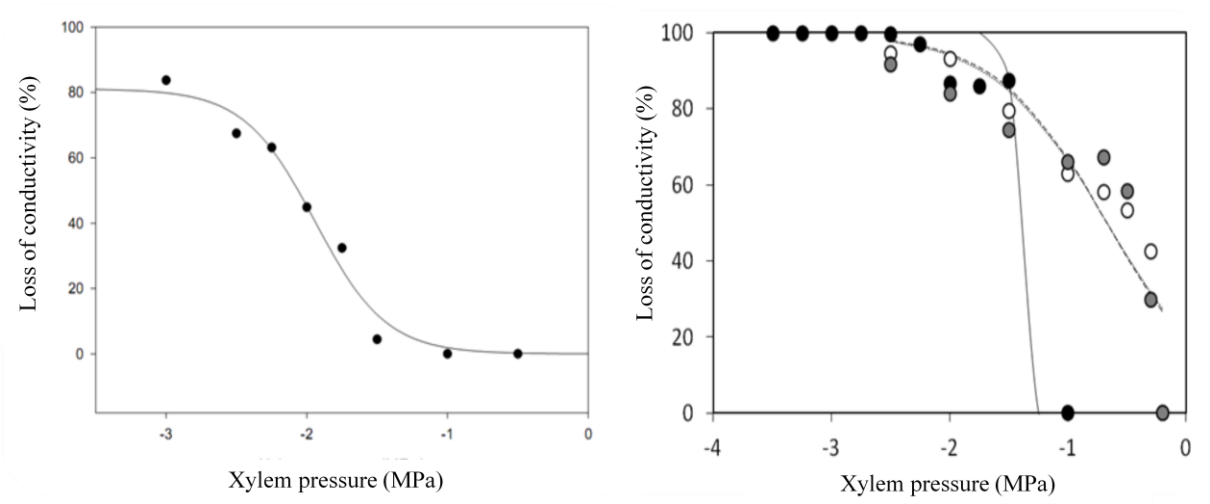


Figure 30. The vulnerability curves (*VC*) obtained from Cavitron technique.

The left panel shows sigmoid *VC* obtained from 0.27 m long branch of PB 260 grown at UBP, Clermont-Ferrand while right panel shows r-shape *VC* measured on 0.37 m long branches of RRIM 600 grown in Thailand.

2.1 *The investigation of xylem vulnerability to cavitation*

Feasibility of the techniques and effects of sample conditions on xylem vulnerability to cavitation

Preliminary studies of the vulnerability to cavitation previously studied using Cavitron gave two completely different results (Figure 30): on one occasion, sigmoid *VC* was obtained from a 0.27-m-long branch of PB 260 grown at UBP, Clermont-Ferrand while r-shape *VC*s were obtained from 0.37-m-long branch samples of RRIM 600 from Thailand. Therefore, the feasibility of using Cavitron for the measurement of xylem vulnerability to cavitation on this species has to verified. Then after, the sample conditions: the age of sample, the exposure to sunlight and the size of sample were tested with Cavitron. In addition, air-injection technique was also tested with this species and compared with the result from Cavitron.

Three rubber clones (PB 235, RRIM 600 and RRIT 251) grown on the field at Surat Thani Rubber Research Center in southern region of Thailand (9˚40'27"N, 99˚6'40"E; altitude 19 m) were chosen for these tests. This plantation is in a traditional cultivation area with 2,333.95 ± 124.30 mm of average annual rainfall and 173 ± 3 days of average number of annual rainy day. For each clone, approximately 1.00 m long branches were randomly harvested from three or four individual trees (n = 20/clone) with the age range of 7 – 10 years old on April, 2012. The branches of RRIM 600 and RRIT 251 clones were all fully exposed to sunlight but differed in age: current year and previous year branches (n = 10 for each age/clone). They were designated for a comparison of the effect of sample age on vulnerability to cavitation and also a comparison of the measurement techniques. Branches of PB 235 were all current year but differed in their exposure to sunlight; half of the samples (n = 10) were fully exposed to sunlight whereas another half (n = 10) were shaded branches. These samples were for the test of the sunlight effect on vulnerability to cavitation.

After the branches were cut from trees, they were immediately defoliated and put in a black plastic bag with moist paper to reduce water loss. They were later cut again to have 0.60 m in length and prepared for shipping to France by wrapped both cutting ends with paraffin, treated with pesticide and stored in sealed plastic bags. When these samples arrived at the laboratory in France, six samples were randomly chosen and immediately measured for

Figure 31. Three rubber plantation sites.

The map indicating rubber plantations which experimentations were took place. Plantation at Surat Thani province is considered in a traditional production area while sites at Nong Khai and Khon Kaen are considered marginal areas for natural rubber production.

Figure 32. Experimental set up at Nong Khai Rubber Research Center for plant responses to drought stress on ten commercial rubber clones.

The experiments were conducted on ten rubber clones: BPM 24, PB 217, PB 235, PB 260, PB 5/51, RRII 105, RRII 118, RRIM 600, RRIT 251 and RRIT 408. Each clone consisted of 10 one-year-old trees, planted in 0.93 m^3 pots filled with 0.83 m^3 sandy loam soil.

native embolism while the rest were rewrapped with moist paper, placed in sealed plastic bag and stored at 4°C up to 10 days before they were used for the analysis of xylem vulnerability to cavitation.

Variation of xylem vulnerability to cavitation

The investigations on genetic variability and phenotypic plasticity of xylem vulnerability to cavitation were carried out using Cavitron technique following a protocol deducted from the previous experiments on the feasibility of the techniques and the sample conditions.

The study on genetic variability of xylem vulnerability to cavitation was conducted on ten rubber clones planted in the field at Nong Khai Rubber Research Center (18˚09'30"N, 103˚09'31"E; altitude 171 m) on March 2013. The average annual rainfall ranging at this location was 1,770.70 ± 96.26 mm with 128 ± 3 of averaged rainy days. This site is considered a marginal area for natural rubber production and under risk of drought stress. For each clone, 6 – 8 branches, at least 0.60 m long, were randomly harvested from one year old trees. They were immediately defoliated and afterward prepared as previously described and then, shipped to France. The defoliated leaves were collected (n = 40/clone) for the measurements of *LMA*.

Phenotypic plasticity of xylem vulnerability to cavitation was studied on two rubber clones (RRIM600 and RRIT251) from three locations: Khon Kaen, Nong Khai and Surat Thani (Figure 31). The plantation at Khon Kaen (16˚48'21"N, 103˚08'22"E; altitude 252 m) is also considered in a marginal production area with the average annual rainfall of 1,256.30 ± 66.87 mm and 109 ± 3 days of rain. Branches from Nong Khai and Surat Thani were harvested from the same locations previously used. For both clones, ten current year branches were harvested from 1 – 2 years old trees from each location on November 2013. Branches were harvested from the sun-exposed part of the canopy. They were prepared and shipped to laboratory in France following the earlier mentioned protocol.

Immediately after branch samples arrived at laboratory in France, branches were randomly chosen for the measurement of native embolism (n = 20 and 12 branches, for the two respective experiments). The remaining branches were rewrapped with moist paper, putted in plastic bag and stored in cold storage (4°C) up to 20 days before they were measured for their vulnerability to cavitation. The features of xylem vulnerability to cavitation (P_{12}, P_{50} and P_{88}) of these samples were later obtained.

Table 5. Environmental conditions at Nong Khai site during November 2012 – March 2013.

	Mean monthly temperature (°C)	Mean RH (%)	Number of rainy days	Total amount of rainfall (mm)	Mean PAR (µmol m^{-2} s^{-1})
Nov 2012	25.42 ± 0.93	70.58 ± 2.43	0	0.00	652.53 ± 136.46
Dec 2012	18.30 ± 0.54	67.76 ± 1.46	0	0.00	715.12 ± 55.28
Jan 2013	21.92 ± 0.64	69.06 ± 1.74	3	26.50	833.47 ± 135.31
Feb 2013	24.93 ± 0.89	63.93 ± 3.16	0	0.00	1,125.08 ± 110.04
Mar 2013	26.13 ± 0.53	61.63 ± 1.34	3	33.60	766.82 ± 266.39

The averaged monthly temperature, relative humidity (RH), number of rainy days, total amount of rainfall and averaged photosynthetically active radiation (PAR) are presented. The data are conducted from local weather station at the NRRC, Nong Khai during drought experiment from November, 2012 to March, 2013.

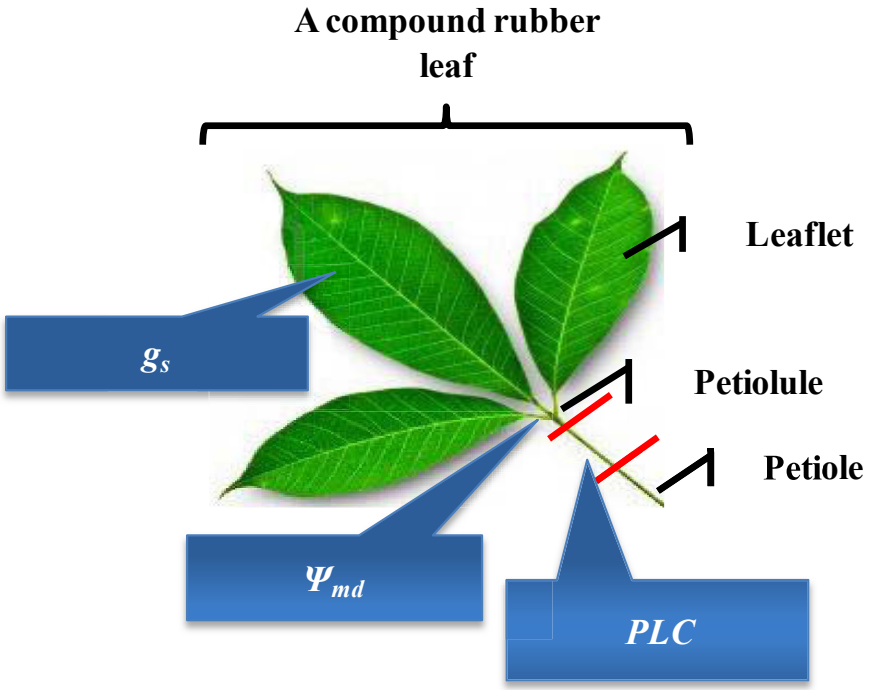


Figure 33 Diagram of a rubber leaf.

A compound rubber leaf consists with three leaflets. The red line indicates a separate point where petiole was cut off the leaf for the measurement of petiole PLC while the rest of leaf was used for the measurement of midday leaf water potential (Ψ_{md}).

2.2 *Plant responses to drought stress*

The experiment on plant response to drought stress was carried out using ten rubber clones (n = 6/clone). Trees were planted in 0.93 m^2 pots filled with sandy loam soil at Nong Khai site on November 11, 2011 (Figure 32). The trees were one year old, watered to full field capacity with a water supply of 15 liters twice a week. This experiment was conducted in a dry season from November, 2012 to March, 2013. During the experiment, the monthly average temperature ranked from 18.30 to 26.13 °C, 61.63 to 70.58 %RH, 3 rainy days in January and March with total amount of rainfall 26.5 and 33.60 mm, respectively while monthly average photosynthetically active radiation (PAR) was ranked from 652.53 to 1,125.08 μmol m^{-2} s^{-1} (Table 5).

Trees were divided into a control and a stress group (n = 3). Because the trees were varied in their size and total leaf area, trees in stress group were selected to be as much as homogenous in their respective characters. On December 7, 2012, water supply was withheld from trees in stress group and the soil was left to dry down for 11 weeks to generate drought stress. During this time, water supply was strictly controlled for all trees. The occasionally rains were prevent to contact with the soil in pots by a transparent coverage. This rain exclusion system was removable; the covers were moved in before the rain and removed afterward to prevent the accumulation of temperature in the pots.

The progress of soil drying was followed by a survey of soil water content (*SWC*, %) along with tree growth and changes in hydric traits. Growth parameters were included tree height and numbers of leaves; they were measured every four weeks. The hydric traits of interest were the midday stomatal conductance (g_s, mmol m^{-2} s^{-1}) the leaf water potential at midday (Ψ_{md}, MPa) and the loss of xylem conductivity (*PLC*, %) in petiole. The measurements of g_s and Ψ_{md} were performed between 12:00 and 14:00. The g_s was randomly measured on a leaflet from three different compound leaves situated on the lowest flush of each tree. Successively after the g_s measurement, one of these compound leaves was covered with plastic bag and removed for Ψ_{md} and petiole *PLC* measurements. A petiole segment at least 0.10 m long was separated from the compound leaf (Figure 33) under water and kept submerged for *PLC* measurement while the rest of leaf was used for Ψ_{md} measurement. The petiole segments were re-cut under water to obtain 0.05 m long segments before they were attached to the Xyl'EM apparatus for the measurement of their embolism.

Table 6. The P_{50} and *FTSWc* values of five apple genotypes selected for this experiment.

Genotype	P_{50}	$FTSW_c$, %
B0023	−2.52	65.0
B0057	−5.84	75.6
B0070	−6.77	45.0
B0097	−3.66	95.0
B0111	−3.70	26.0

The 50% loss of xylem conductivity (P_{50}) and the critical fraction of transpirable soil water ($FTSW_c$) are presented. The P_{50} indicates cavitation resistance thus, the safety of xylem water transport and the $FTSW_c$ indicates sensitivity of stomatal regulation to drought stress.

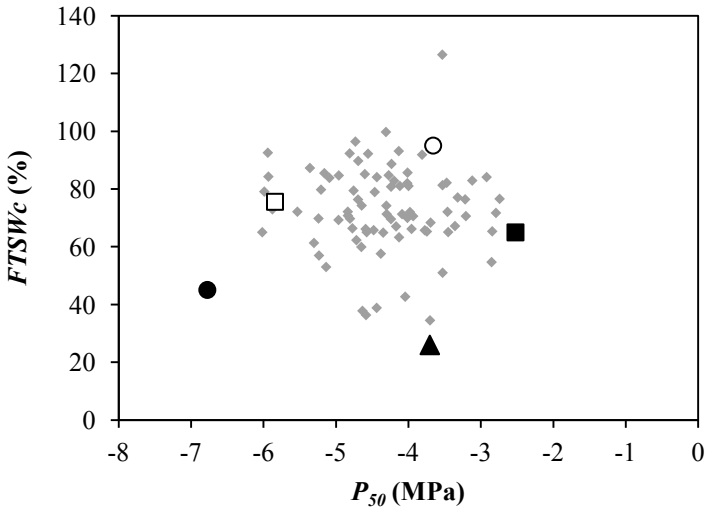


Figure 34. Variation in hydraulic efficiency ($FTSW_c$) and safety (P_{50}, MPa) of 122 apple genotypes.

A progeny derived from 'Starkrimson' x 'Granny Smith'. Five selected genotypes: B0023 (■), B0057 (□), B0070 (●), B0097 (○) and B0111 (▲) are presented.

Due to leaf shedding and different number of leaves on the lowest flush of the trees, when there was no more leaf left for the g_s measurement on a tree, the measurement was moved up to a higher flush for all trees. The relative stomatal conductance ($g_s/g_{s\,max}$, %) was calculated using the midday stomatal conductance from stressed trees (g_s) and the midday stomatal conductance averaged from the control trees ($g_{s\,max}$) measured on the same day.

Afterward, the $g_s/g_{s\,max}$ was plotted versus Ψ_{md} and fitted with sigmoid function according to equation *14* to calculate water potential causing 12, 50 and 88% of stomatal closure ($g_{s\,12}$, $g_{s\,50}$ and $g_{s\,88}$). The petiole vulnerability to cavitation was also obtained by plotting the petiole *PLC* versus Ψ_{md}. The features of petiole vulnerability to cavitation (P_{12}, P_{50} and P_{88}) and the relative shed leaf area, the xylem tensions causing 12, 50 and 88 percent of leaf shedding (LS_{12}, LS_{50} and LS_{88}, respectively) were calculated and the safety margins of petiole and branch were later calculated.

3. Apple trees

Five genotypes (B0023, B0057, B0070, B0097 and B0111) out of 122 genotypes available from a progeny of 'Starkrimson' x 'Granny Smith' were selected for this study. The progeny is characterized by a strong variability of tree vigor and architectural traits (Segura *et al*., 2008; Segura *et al*., 2006; Segura *et al*., 2007). The progeny has been studied for their hydraulic safety and water saving behavior (Lauri *et al.*, 2011; Regnard *et al.*, 2009). We chose the five genotypes from their contrast characters in hydraulic safety and water saving behavior which were representing by the pressure causing 50 percent loss in xylem conductivity (P_{50}, MPa) and the critical fraction of transpirable soil water ($FTSW_c$, %) which was soil water content that the transpiration rate of tree started to decline, respectively (Figure 34). The P_{50} of five genotypes ranked from −2.52 to −6.77 MPa while it was −3.05 and −5.70 MPa for the parents: 'Starkrimson' and 'Granny Smith', respectively while the $FTSW_c$ of five genotypes ranked from 26.0 to 95.0% with value of 70.0 and 81.0% for the parents 'Starkrimson' and 'Granny Smith', respectively (Table 6).

The selected genotypes were grafted on M9 rootstocks by professional breeder (n = 40/genotype). They were planted in ten-liter-pots filled with mixed loam soil on March 2012 at INRA site Crouël, Clermont-Ferrand (Figure 35). They were grown in a greenhouse under the average temperature of 17.09 ˚C, 65.95% RH and average PAR of 105.47 µmol photons

Figure 35. Five apple genotypes grown in greenhouse at INRA site Crouël, Clermont-Ferrand.

The five genotypes: B0023, B0057, B0070, B0097 and B0111 (n = 40/genotype) were grown under optimal condition in the greenhouse for three months before drought experiment was begun in 2012.

$m^{-2}s^{-1}$ for three months. During this period, they received 0.50 liter of water through drip irrigation every two days. These trees were used for the measurement of vulnerability to cavitation and the plant response to drought stress during the growing season of 2012 and 2013.

3.1 *The xylem vulnerability to cavitation*

The xylem vulnerability to cavitation was measured with Cavitron on non-stress stem samples. This measurement aimed to verify the intraspecific variation previous reported on vulnerability to cavitation from the study of Lauri *et al.* (2011) since this trait is known to vary not only by genetic control (Cochard *et al.*, 2007; Lauri *et al.*, 2011; Vander Willigen and Pammenter, 1998) but also by the influence of environmental conditions (Choat *et al.*, 2007; Cochard *et al*., 1999; Corcuera *et al*., 2011; Herbette *et al*., 2010; Plavcová and Hacke, 2012) or by the combination of both.

On July, 2012, 4 – 5 trees/genotype were harvested for the measurement of xylem vulnerability to cavitation using Cavitron technique. Trees were at least 0.75 m in height when they were cut. The harvested stem was divided into two parts: the distal part was used for native embolism measurement while the proximal part was used for measurement of vulnerability to cavitation. By plotting the *PLC* with xylem tension and fitted the data with sigmoid function, the features of vulnerability to cavitation (P_{12}, P_{50} and P_{88}) were obtained. All leaves from these harvested trees were collected and measured for leaf area (*LA*), in addition, 30 leaves/ genotypes were used for *LMA* measurement.

3.2 *The response to drought stress*

Plant response to drought stress was conducted in two growing seasons of 2012 and 2013. For the experiment in 2012, the trees were divided into two groups: control (n = 6/genotype) and stress (n = 30/genotype) for the investigation of plant response to a drought stress. We covered soil surface with white plastic to minimize water loss from soil evaporation and therefore, water loss can be assumed as plant transpiration.

The traits of interest for this experiment included: *SWC* (%), tree growth (stem length, number of leaves and stem diameter) and hydric traits (g_s, Ψ_{md} and stem *PLC*). We select three homogenous trees from each genotype and each treatment to follow the changes in tree growth, *SWC* and g_s throughout the experiment. The measurements of Ψ_{md} and stem *PLC*

were conducted on the other remained treatment trees (n = 3/each measurement). After the reference measurement of the interested traits were done, drought stress was applied to trees in stress group by completely withholding the irrigation on July 20, 2012 and the soil was left to dry down for 31 days. Stem length, number of leaves and stem diameter were recorded every ten days while pot weight and hydric trait (g_s and Ψ_{md}) were measured twice a week for both control and stressed trees. The time to harvest stems for *PLC* measurement was decided according to Ψ_{md}; when Ψ_{md} reached the values of interest predicted by vulnerability curve. The g_s was measured between 11:00 and 13:00 on three tagged leaves of each tree; these leaves were located at a relatively similar level on all trees. The Ψ_{md} was successively measured on a random leaf/tree which located close to the leaves used for g_s measurement. If the Ψ_{md} of interest was reached; stems were consecutively harvested for *PLC* measurement. Stem was cut at 0.05 m above the grating point, put in moist plastic bag and immediately brought into laboratory. Then after 0.10 m long segment was cut from the center of the stem and it was measured for *PLC*; the cutting was done under water to prevent air to infiltrate the cut segment. After the measurement on day 31 of drought stress, irrigation was resumed for the harvested trees at the rate of 0.50 liter of water every two days. They were kept in greenhouse with optimal cultivation practices for the experiment in 2013.

For the experiment in 2013, only the regrown trees from stress group were used (n = 18/genotype). On March 2013, new irrigation scheme, 0.25 liters of water/tree, was applied twice a week; lower irrigation rate was expected to enhance different response to drought stress of the five genotypes. They were grown with this new irrigation scheme for four months then trees were divided into two groups: a control (n = 3/genotype) and a stress (n =15/genotype). Three most homogenous trees from stress group were selected for the measurements of tree growth traits, *SWC* and g_s throughout the experiment. The experiment was conducted following a similar protocol used in 2012. After reference measurements, irrigation was completely withheld for trees in stress group and the soil was left to dry down for 33 days from August, 2013.

The relative stomatal conductance ($g_s / g_{s\ max}$, %) was calculated using the midday stomatal conductance from stressed trees (g_s) and the midday stomatal conductance averaged from the control trees ($g_{s\ max}$) measured on the same day. Afterward, the $g_s / g_{s\ max}$ was plotted versus Ψ_{md} and fitted with sigmoid function according to equation *14* the features of stomatal regulation ($g_{s\ 12}$, $g_{s\ 50}$ and $g_{s\ 88}$) were obtained and safety margin was calculated.

II. Methods

1. The measurements of xylem vulnerability to cavitation

The xylem vulnerability to cavitation was measured with Cavitron technique (Cochard, 2002b; Cochard *et al*., 2005) or air-injection technique (Ennajeh *et al*., 2011). Before samples were measured for this trait using the two techniques, they were checked for the presence of open vessels using air infiltration technique (Ewers and Fisher, 1989; Zimmermann and Jeje, 1981). After branches were cut in the air to obtain the desired length, they were successively infiltrated with compressed air (0.10 MPa) at one end while the other end was submerged under water. Because compressed air cannot pass through vessel end walls, the air bubbles observed are indicated that the present of open to center vessels. Only samples with intact vessels (no air bubble observed) were used for the measurement of xylem vulnerability to cavitation with Cavitron or air-injection technique.

1.1 *Cavitron*

The xylem tension (P) on Cavitron was firstly set to a reference pressures (−0.75 or −1.00 MPa) and the K_{max} was determined. Then, the tension was increased stepwise with −0.25 or −0.50 MPa increment and new sample conductance (K_i) was determined. The *PLC* was computed according to the equation *17* and the procedures were repeated until more than 90% *PLC* was obtained. The *VC* was later fitted with the sigmoid function (Pammenter and Van der Willigen, 1998) as indicated in equation *14*. The P_{50} and s, the slope of *VC* were determined from the fitting then, P_{12} and P_{88} were calculated according to the equation *15* and *16*. The P_{12} is considered as the "air entry point" which the embolism begins (Sparks and Black, 1999) while P_{88} is the tension before xylems becomes totally non-conductive or "full embolism point" (Domec, 2001).

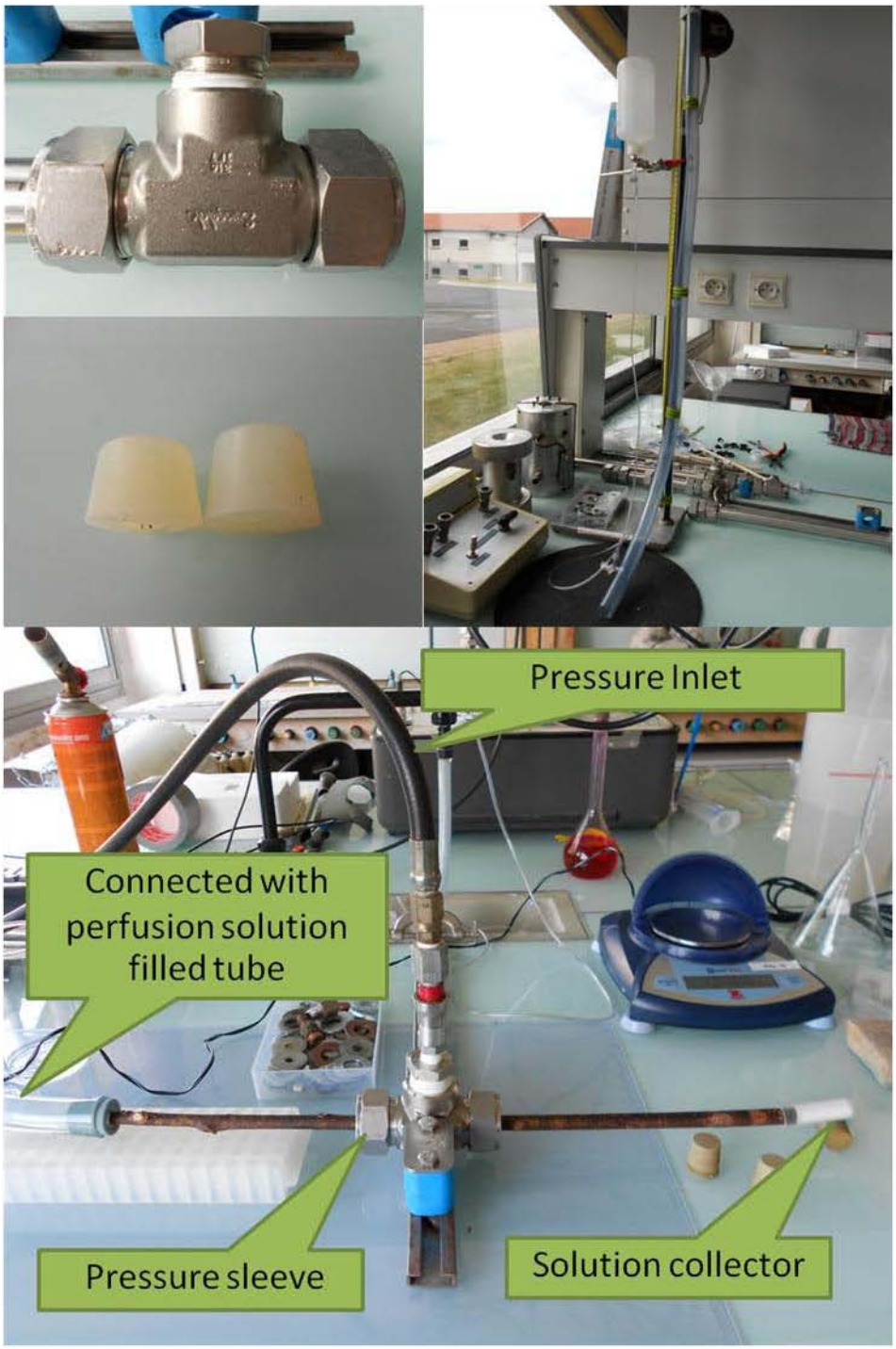


Figure 36. The supplies used in air-injection technique and the set up for measuring xylem vulnerability to cavitation on a rubber branch.

The figure presents: a steel pressure sleeve, rubber corks for sealing sample with the sleeve, the perfusion tube and the set up for the measurement. Barked was peeled off at the center of segment and then inserted into the sleeve. Its proximal end was attached to the perfusion solution filled tube and a tube with cotton was attached to its distal end to collect the solution that flow through sample. The tube with cotton was weight for the calculation of hydraulic conductance.

1.2 *Air-injection*

The pressure sleeve was made with a 7.50-cm-long three-ways stainless steel tube with a diameter of 2.50 cm from Swagelok Company. A branch segment was prepared by re-cut both ends to obtain a desired length (0.37 m long) in the air. Bark was peeled off at the center of the branch where pressure sleeves will be placed; this will allow the air to enter the vessels and induce xylem embolism. The portion of the segment in contact with compressed air inside the chambers was 3.5 cm. The branch was then inserted into the sleeves with compression fittings placed at both ends of the tube. Rubber corks were used to seal the sleeves and the sample (Figure 36).

Air pressure in the sleeves was firstly set at reference level (1.00 MPa) and hydraulic conductance (K) of sample was calculated as:

$$K = \frac{F}{P} \qquad (19)$$

where F and P were the flow rate through sample and the pressure in perfusion solution filled tube that connected to distal end of sample, respectively. The F was calculated from the weight of solution collected for 1 minute from the proximal end of branch segment. The *PLC* was calculated using equation *17* and air pressure in chamber is increased by 0.50 MPa stepwise until more than 90% *PLC* was obtained. The *VC* was constructed by plotting *PLC* versus P then fitted with sigmoid curve using equation *14*. The P_{12}, P_{50} and P_{88} were then obtained.

2. Measurements of plant hydric traits

The hydric traits of interest: the soil water content, the water potential, the stomatal conductance and the xylem embolism were measured. Afterward, the features of stomatal regulation to drought stress and xylem vulnerability to cavitation as well as the safety margin of the interested organs were calculated.

2.1 *The soil water content*

The progression of drought was followed through the change of soil water content which was calculated as:

$SWC = 100 \times (\text{fresh weight} - \text{dry weight}) / \text{dry weight}$ *(20)*

In the experiment on rubber tree, the soil was sampled from each pot at two depths from soil surface: 0.30 and 0.60 m. The cylindrical soil core sampler was used to obtain 101.38 cm^3 of the soil from each depth. They were later mixed and weight for soil fresh weight, oven-dried at 105˚C for 24 hours then after, soil dry weight was measured and *SWC* was calculated. The sampled soil was returned to the pots afterward to maintain soil volume in the pots.

For apple tree, *SWC* was calculated from the mass of whole pot. After irrigation was given in the morning and the exceeded water was left to drain off for two hours, pots were weighted on digital balance (n = 3/genotypes) to obtain a fresh weight of the soil. Similar soil mixture and at the same amount which was used to fill pots was oven-dried for 48 hours. It was later weight for a dry weight. To facilitate the measurement, both soil masses were included pot weight.

2.2 *Leaf water potential*

In succession of the g_s measurement, leaves were removed from the trees for Ψ_{md} measurement. Before the leaf was removed, it was covered with a plastic bag along with its petiole. The pressure chambers: Plant water status console model 3005F1 (Soilmoisture Equipment Corp., CA, USA) and Model 1505D Pressure chamber (PMS Instrument, OR, USA) for experiment on rubber and apple trees, respectively.

2.3 *Stomatal conductance*

Midday stomatal conductance (g_s, mmol m^{-2} s^{-1}) was measured using the porometer AP4 Delta-T (Cambridge, U.K.) for experiment on rubber trees and the porometer Li-1600 Steady State (LI-COR, Nebraska, USA) for experiment on apple trees. These measurements were conducted only under full sun condition during the midday from 12:00 to 14:00 and from 11:00 to 13:00 for rubber and apple trees, respectively.

Relative stomatal conductance ($g_s/g_{s\ max}$, %) was later calculated using stomatal conductance of stressed tree (g_s) and averaged stomatal conductance of the control trees ($g_{s\ max}$) on the same experimental day. The $g_s/g_{s\ max}$ allows a better comparison of stomatal regulation between trees by reducing the variation of g_s from environmental fluctuation between the measurements.

In order to find the relationship between stomatal closure and the reduction in plant water potential, the $g_s/g_{s\ max}$ was plotted versus Ψ_{md}. Data was later fitted with sigmoid function according to equation *14* to obtain water potential causing 50 percent of stomatal closure ($g_{s\ 50}$) and *s* value. Then after, water potentials causing 12, and 88 percent of stomatal closure ($g_{s\ 12}$ and $g_{s\ 88}$ respectively) were calculated as:

$$g_{s12} = g_{s50} + \frac{50}{s} \qquad (21)$$

$$g_{s\,88} = g_{s50} - \frac{50}{s} \qquad (22)$$

2.4 *Xylem embolism*

The embolism rate on petioles or branches was measured with Xyl'EM, embolism meter following a procedure described by Cruiziat *et al.* (2002). The long sample was re-cut under water and the segment with a desire length (0.10 m long for branch or stem samples and 0.05 m long for petiole samples) was collected from the middle part of sample. The proximal end of segment was then attached to the hydraulic apparatus and K_i of sample was firstly measured. Segment was then flushed with pressurized solution of KCl 10 mM and $CaCl_2$ 1 mM for five minutes to eliminate formed embolism in the conduits. The flushing is repeated until the *K* measured was stable and the K_{max} was determined. The *PLC* was calculated according to equation *17*.

In this study, xylem embolism may be referred as a native embolism or just the *PLC*. For the native embolism, it is a prerequisite measurement proposed and explained by Awad *et al.* (2010). Before the measurement of xylem vulnerability to cavitation, representative branch or stem samples were selected for this measurement. Low and rather homogenous native embolism was expected from these samples. The xylem vulnerability to cavitation was only measured on the conductive vessels. Therefore using sample with high native embolism rate may overestimate its vulnerability to cavitation since the remained functioning conduits were likely to be more cavitation resistance. The *PLC* was measured on petioles of rubber tree and stems of apple trees. This trait was measured through the progression of drought stress for the respective species.

2.5 *Safety margin*

Plant hydraulic safety margin can be determined using different threshold tensions related to safety and efficiency of xylem transport (Choat *et al*., 2012; Markesteijn *et al*., 2011; Meinzer *et al*., 2009). In this study, the hydraulic safety margin of rubber and apple trees studied was calculated according to following hypotheses: (*i*) stomatal regulation should occur to prevent the critical embolism in petiole and (*ii*) if stomatal regulation fails to prevent high embolism rate in petiole, the embolism should induce leaf shedding and therefore prevent loss of hydraulic conductivity in critical organs (branches and stems).

In the drought experiment of rubber tree, safety margin of petiole and branch were calculated from $g_{s\ 50}$ and LS_{50} with P_{50} of the respective organs while for apple tree, the safety margin of stem was calculated from $g_{s\ 50}$ and P_{50} of stem.

3. Measurements of leaf and growth traits

Leaf area (LA, m^2) was measured using leaf area meter LI-3000A (LI-COR inc.) and the size of individual leaf (cm^2) was estimated. Some of these leaves were then dried at 70˚C for 48 hours in a gravity convection oven, weighted for their dry weight and the leaf mass per area (LMA, g m^{-2}) was calculated as following equation:

$$LMA = leaf\ dry\ weight\ /\ LA \qquad (23)$$

During the experiment of drought stress on rubber and apple trees; changes in stem height, diameter and total leaf area were measured as the indicator for drought sensitivity. The total leaf area was estimated from the size of individual leaf and number of leaves at a given time.

In addition, we used the total leaf area of rubber tree to calculate a relative shed leaf area (LS, %), the drought-induced shed leaf area to the averaged shed leaf area of the control trees. It is necessary to use the relative value for a comparison since leaf shedding was observed on both control and stressed trees during drought experiment. Afterward, LS was plotted against Ψ_{md} and fitted with sigmoid function (Pammenter and Van der Willigen, 1998) in equation *14* then, LS_{50} and s were obtained. The LS_{12} and LS_{88} were calculated following these equations:

$$LS_{12} = LS_{50} + \frac{50}{s} \qquad (24)$$

$$LS_{88} = LS_{50} - \frac{50}{s} \qquad (25)$$

Stem height was measured for rubber and apple trees then the relative growth rate (*RGR*), the change in relative growth over time, was calculated from the logarithm of tree height over drought stress experiment for both species. Changes in stem diameter were recorded for the apple trees during the experiment of drought stress.

4. Measurements of wood density and wood anatomical traits

4.1 *Wood density*

Wood density or wood infradensity (*WD*, g cm^{-3}) was measured on segments from the proximal part of samples used in the measurement of xylem vulnerability to cavitation. The 3 cm long segment was firstly separated; it was cut again in half lengthwise and the pit was eliminated. Because this measurement was carried out on the frozen wood samples, it is necessary to rehydrate the sample by placing them in de-ionized water under vacuum for at least an hour. Fresh volume (V_s, cm^3) of the segments was obtained by a water displacement method on an analytical balance according to the principle of Archimedes. Then the segments were oven-dried at 70˚C for 2 days and their dry weights (M_0) were measured. The *WD* was calculated as the following equation:

$$WD = M_0 / V_s \qquad (26)$$

4.2 *Wood anatomical traits*

In this study, wood anatomical traits were observed on branch samples of six Persian walnut cultivars studied. Two to three 20-lm-thick cross sections were taken using a sledge microtome from the central part of branch segments used for vulnerability to cavitation measurement. The cross sections were bleached and rinsed with water, stained for 30 seconds with a 1:2 mixture of Safranin (0.5 percent in 50 percent ethanol) and Astra Blue (1 percent in water), dehydrated in an ethanol series (50, 75 and 96 percent) and mounted on microscope slide.

The cross sections were observed under an optical microscope (X 20) and photographed with a digital camera (AxioCam HR, Zeiss). Wood anatomy was deciphered by image analysis using ImageJ software (Rasband, 1997-2009). The vessels were isolated by automatic

segmentation then their diameters (µm), density (number mm^{-2}) and the lumen vessel area (A_v, %) were measured and calculated.

III. Statistical analysis

The analysis of variance (ANOVA) was used to test the effects of interested parameters on the xylem vulnerability to cavitation. These parameters included sample conditions: length, age, light exposure and water availability in plantation, as well as direction of water flow through sample while spinning on Cavitron and genetic variation of the studied species. The traits related to plant response to drought stress: *SWC*, Ψ_{md}, $g_s / g_{s\ max}$, *PLC*, *LS* and safety margin were also analyzed. When significant differences were found, Duncan's multiple range test or Tuekey-Kramer Multiple Comparison with *P*-value < 0.05 were used.

RESULTS AND DISCUSSION

I. Is there any variability for xylem vulnerability to cavitation resistance in walnut trees (*Juglans* spp.)?

II. Clonal variability for vulnerability to cavitation and other drought related traits in *Hevea brasiliensis* Müll.Arg.

III. Genetic variation in plant response to drought stress among five apple genotypes from a progeny of 'Starkrimson' x 'Granny Smith'

IV. General discussion

This chapter is divided into two sections: the result and discussion for each species and the general discussion for this whole work. Firstly, the results are presented and discussed successively the experiment on the three species. Experiments on walnut and rubber trees are submitted to publication and they are thus presented in a format ready to publish. The results of experiments on apple tree are divided into two sections: the variation in xylem vulnerability to cavitation and the responses of tree to drought stress. Following these results is the discussion for the apple tree.

Then, in a second part of this chapter, the results obtained are integrated in a general discussion about the variability for the xylem vulnerability to cavitation in agronomic species and the potential of this trait in breeding program as a trait for drought tolerance screening.

I. Is there any variability for xylem vulnerability to cavitation resistance in walnut trees (*Juglans* spp.)?

Abstract

Introduction

Materials and methods

1. Plant materials
2. Leaf mass per area (LMA)
3. Native embolism
4. Estimation of maximum vessel length
5. Xylem vulnerability to cavitation
6. Wood density and wood anatomical traits
7. Statistics

Results

1. Methodological analysis on the measurement of vulnerability to cavitation
2. Xylem vulnerability to cavitation of Persian and hybrid walnuts
3. Wood density and wood anatomical traits

Discussion

Reference

Is there any variability for xylem vulnerability to cavitation resistance in walnut trees (*Juglans spp.*)?

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Abstract

Drought tolerance selection is a current challenge for breeding program in an attempt to ensure agricultural productivity especially under the global context of increasing intensity and frequency of drought. In tree species, xylem vulnerability to cavitation is among the most important traits related to drought-induced mortality. It can be rapidly evaluated, hence ensuring large-scale screening for drought resistance. Genetic variability and phenotypic plasticity for this trait are well studied in natural populations; however, it is yet to be studied for cultivated tree species. In this work, genetic variability in xylem vulnerability to cavitation of six Persian walnuts (*Juglans regia* L.) and six hybrid walnuts (*J. regia* x *J. nigra*) was investigated. In addition, methods for measuring xylem vulnerability to cavitation using the centrifuge technique 'Cavitron' were evaluated in order to improve the accuracy of the technique. The Cavitron technique was found suitable to assess xylem vulnerability to cavitation on this species using 0.38 m rotor which is large enough to analyze samples with intact vessels. Despite differences wood anatomical traits, xylem vulnerability to cavitation among Persian walnuts studied was similar. Very narrow variations on xylem vulnerability to cavitation were also found among hybrid walnuts and only slightly differences were observed when compared the two species even though previous studies have shown differences in various traits among these species. These results suggested the uniform-selection which leading to canalization in cavitation resistance for cultivated *Juglans*.

Keywords: vulnerability to cavitation, drought stress, Cavitron, *Juglans* spp.

Introduction

The importance of water resource management grows vastly while agriculture is the largest user of this resource. One of the concerns for water resource management is to improve the agricultural water use. In addition, drought has been predicted to occur more frequently and become severer (IPCC, 2014). In this context and to ensure crop productivity, the selection for drought tolerance crops is one the main challenge, especially for perennial crops.

Long-distance water transport takes place in xylem conduits under negative pressure. The ascent of water is driven by evaporative force (Dixon and Joly, 1894). Even though the tension is necessary to bring up water, the great tensions that develop in xylem conduits due to drought stress can be critical to plant survival. Water in conduits is in a metastable state and susceptible to cavitation (Tyree and Sperry, 1989). The expansion of air bubble can fill the conduit and embolized it, leading to a loss of xylem conductance (Zimmermann, 1983). Studies showed that xerophilous species have higher resistance to xylem cavitation than hygrophilous ones (Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003). The vulnerability to cavitation is then considered as a critical trait for drought tolerance of woody species (Choat *et al.*, 2012).

Vulnerability to cavitation is an intrinsic property of the conductive elements of the xylem (Cochard, 2006; Tyree *et al.*, 1994; Tyree and Ewers, 1991) and can be evaluated without submitting the plant material to a long period of drought. This trait was found to vary at the intra-specific level in many forest tree species (Corcuera *et al.*, 2011; Herbette *et al.*, 2010; Maherali and DeLucia, 2000; Martínez-Vilalta *et al.*, 2002). The variation in this trait can be due to genetic difference (Cochard *et al.*, 2007; Wortemann *et al.*, 2011) and/or phenotypic plasticity (Awad *et al.*, 2010; Corcuera *et al.*, 2011; Herbette *et al.*, 2010). This trait thus appears to be a relevant criterion for screening genotypes for drought tolerance. For forest trees, the genetic diversity was mainly found within populations (Lamy *et al.*, 2011; Wortemann *et al.*, 2011) with few differences between populations, indicating that this trait is genetically canalized for natural populations (Lamy *et al.*, 2011). The genetic variability for vulnerability to cavitation needs to be further tested on cultivated fruit trees. First, they are submitted to different selection pressures than natural populations, and they are not submitted to natural selection pressures that could reduce the variability. Second, the effect from selecting for yield on vulnerability to cavitation is unclear since there are contrasting data on

the relationship between yield traits and vulnerability to cavitation (Cochard *et al.*, 2007; Lamy *et al.*, 2011).

Walnuts (*Juglans* spp.) are economical important tree species. The most important species are the Persian walnut (*J. regia* L.) and the eastern black walnut (*J. nigra* L.) which are cultivated for nut and wood production in America, Europe and Asia. The important cultivation areas are included in Mediterranean, semiarid and arid regions (Aletà *et al.*, 2009; Vahdati *et al.*, 2009) that can be considered as marginal production areas for this species in term of water availability. Since walnut requires large amount of water to ensure optimal growth and productivity (Fulton and Buchner, 2006), irrigation is needed for this crop species (Reid *et al.*, 2009). To date, few studies have been carried out to investigate genetic and phenotypic variability in physiological responses of this genus to environmental factors, especially responses to drought stress under the context of climate changes (Gauthier and Jacobs, 2011).

Walnut is considered as a drought avoidant (Lucier and Hinckley, 1982). It displays several desiccation avoidance mechanisms such as deep root system (Pallardy and Rhoads, 1993), stomatal sensitivity to relative humidity (Cochard *et al.*, 2002), and drought-induced leaf abscission (Tyree *et al.*, 1993). These traits help to prevent extensive cavitation development during water stress. Previous study of Aletà *et al.* (2009) pointed out the intraspecific variation in water use efficiency among Persian walnut genotypes which suggested that there is a difference in drought responses among the genotypes. In addition, a study of Vahdati *et al.* (2009) also demonstrated differences in drought response between Persian walnuts for germination and growth. However, there is no insight on the genetic variability for drought tolerance traits, especially for vulnerability to cavitation.

In this study, we evaluated the genetic diversity of vulnerability to cavitation in *Juglans* spp. First, we tested several methods for measuring the vulnerability to cavitation in order to improve the accuracy of vulnerability curves in this tree species. Then, we investigated this trait in six cultivars of *Juglans regia* and six hybrids *J. regia* x *J. nigra* in relation with xylem structural traits. We hypothesized that the vulnerability to cavitation is much higher for hybrids than for commercial cultivars, as the variability could be enhanced by inter-specific cross (Cochard *et al.*, 2008).

Materials and methods

1. Plant materials

The measurements on vulnerability to cavitation on Persian walnuts were conducted on 15 – 20 years old walnut trees grown outdoors at INRA site Crouël at Clermont-Ferrand in south-central France (45°46′27″N, 3°8′36″E; altitude 338 m). They were comprised of 48 individuals trees from 6 cultivars; Chandlers (Ch, 4 individuals), Fernettes (Ft, 3), Fernors (Fo, 4), Franquettes (Fq, 17), Laras (La, 15), and Serrs (Se, 4). Seventeen branches were randomly sampled in order to verify the analytical technique with Cavitron, on October 2011. Then, three to six branches were collected from each studied trees to investigate the level of vulnerability to cavitation among the cultivars, on November 2011. Selected branches were current year, mature, from the sun-exposed part of the canopy and with at least 0.45 m in length. Branches were immediately defoliated and four representative leaves were collected for the analysis of leaf mass per area (*LMA*). Twenty branches were randomly chosen from these samples for native embolism measurement while the rest were stored in cold storage (4°C) up to 30 days before the analysis of their xylem vulnerability to cavitation.

Furthermore, 0.60-1.00 m long branches were collected from six wood-oriented hybrid walnuts (*J. regia* x *J. nigra*): F1, F2, H3, J3, N1 and N3 on November 2013. For each hybrid, current year branches (n = 8 to 10) were collected from sunlight exposed part of two individual trees. The sampling was carried out as mentioned before.

2. Leaf mass per area (*LMA*)

Four leaves from each stem of Persian walnuts used for the analysis of xylem vulnerability to cavitation were measured for leaf mass per area (g m^{-2}). Leaf area was determined with leaf area meter LI-3000A (LI-COR inc.) and dry mass was measured on leaves after drying at 70˚C for 48 hours in a kiln.

3. Native embolism

Native embolism was measured with Xyl'EM following the procedure described by Cruiziat *et al.* (2002) on branches of both Persian (n = 15) and the hybrid (n = 12) walnuts. This prerequisite was proposed and explained by Awad *et al.* (2010). A 0.10 m-long segment was

cut under water from the center of the collected branches, and then its proximal end was attached to the hydraulic apparatus. Initial hydraulic conductance (K_i) was measured under low pressure (6 − 9 kPa) using a solution of KCl 10 mM and $CaCl_2$ 1 mM. Then, after the segments were perfused with the same solution at 0.15 MPa to remove embolism, maximum hydraulic conductance (K_{max}) was determined under low pressure. The percentage loss of xylem conductivity (*PLC*) was calculated as followed:

$$PLC = 100 \times (1 - \frac{K_i}{K_{\max}}) \qquad (1)$$

4. Estimation of the maximal vessel length

Cut open vessels on sample have been known to be more sensitive to cavitation than intact vessels and can yield abnormal r-shape vulnerability curve when measuring xylem vulnerability to cavitation using Cavitron (Cochard *et al.*, 2010). Before branch segments were subjected to the measurement of xylem vulnerability to cavitation with this technique, they were checked for the presence of open vessels using air infiltration technique (Ewers and Fisher, 1989; Zimmermann and Jeje, 1981). After branches were cut in the air to obtain the desired length (0.28 and 0.38 m long), they were successively infiltrated with compressed air (0.10 MPa) at each end while another end was submerged under water. Air bubbles were observed when vessels were cut open.

5. **Xylem vulnerability to cavitation**

The xylem vulnerability to cavitation was measured with the Cavitron (Cochard, 2002; Cochard *et al.*, 2005). The principle of this technique is to use centrifugal force to increase the tension in the water columns while measuring the decrease of hydraulic conductance. Branches were prepared by peeling the barks at both ends and cutting in the air to obtain a fixed length. We first used 0.28 and 0.38 m long branches on Persian walnuts in order to test the effect of sample length on the measurements. Then, the following measurements were performed on 0.38 m long segments. We also tested the effect of the water flow direction on the measurements (sense and antisense flows). During the spinning on the Cavitron, water flows from upstream reservoir where there is larger amount of water to downstream reservoir (Cochard, 2002). The flow direction was controlled by the relative position of the reservoirs

at the sample ends. The flow from proximal to distal end of sample is called sense flow while the flow from distal to proximal end is the antisense flow. Xylem pressure (P) was, at first, set to a reference pressure (−0.75 MPa) and the K_{max} was determined. The xylem pressure was then set to more negative pressure and the new sample conductance (K_i) was determined. The procedure was repeated for more negative pressures (with −0.25 or −0.50 MPa increments) until *PLC* reached at least 90%. *PLC* of the stem was computed according to the eq. 1. After measurement, samples were stored at −30°C for further analysis on wood anatomy. The vulnerability curve (*VC*) was later constructed by plotting *PLC* versus xylem water tension. For each curve, the raw data were fitted using the sigmoid function (Pammenter and Van der Willigen, 1998):

$$PLC = \frac{100}{1 + e^{\left(\frac{s}{25 \times (P - P_{50})}\right)}} \qquad (2)$$

Where P_{50} (MPa) is the pressure causing 50% loss of xylem conductivity and s is the slope of *VC*. The xylem water potential causing 12 and 88% loss of xylem conductivity (P_{12} and P_{88} respectively) were calculated according to the eq. 3 and 4 as follows:

$$P_{12} = P_{50} + \frac{50}{s} \qquad (3)$$

$$P_{88} = P_{50} - \frac{50}{s} \qquad (4)$$

The P_{12} is considered as the "air entry point"; which the embolism begins (Sparks and Black, 1999) while P_{88} is the "full embolism point" which is the tension before xylem becomes totally non-conductive (Domec and Gartner, 2001).

6. Wood density and wood anatomical traits

Wood infradensity (*WD*, g.cm^{-3}) was measured for each sample on two segments from the proximal part of the samples used for xylem vulnerability analysis. The *WD* was determined by the following equation:

$$WD = \frac{M_0}{V_s} \qquad (5)$$

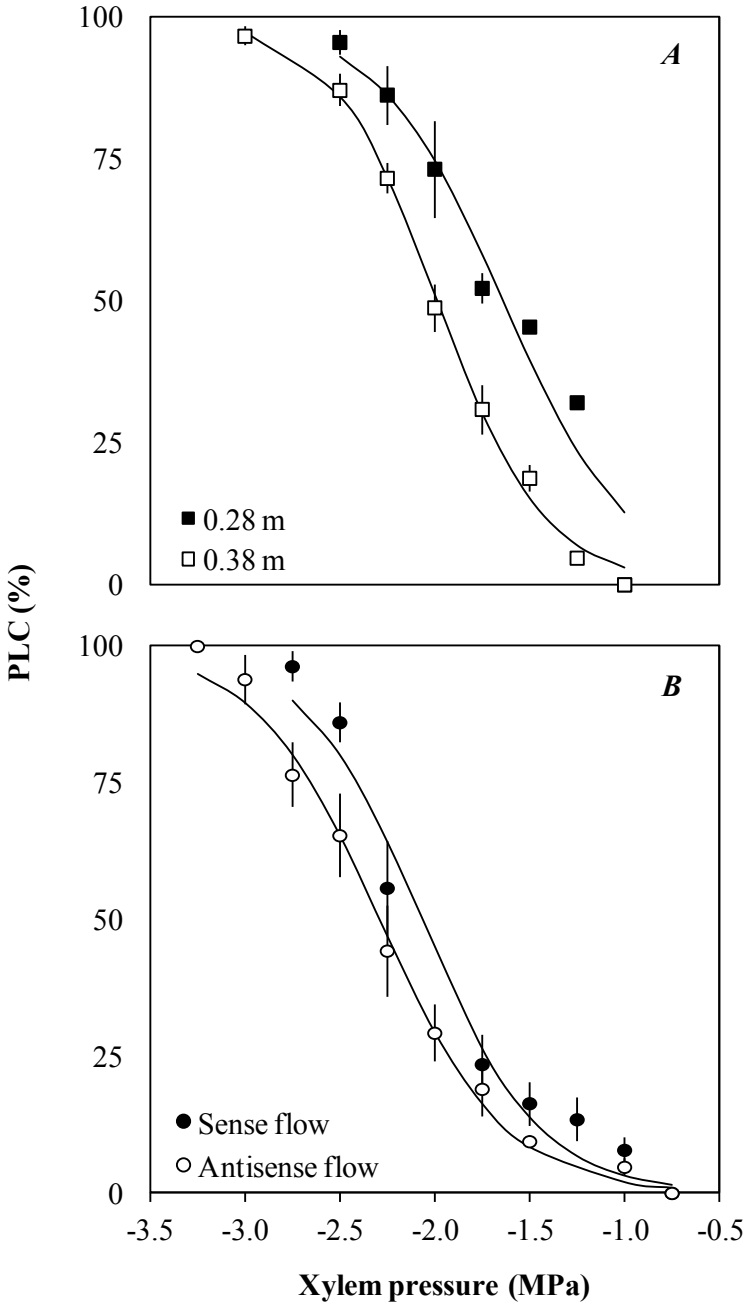

Figure 1. Effects of sample size (*A*) and water flow direction (*B*) on the xylem vulnerability to cavitation of walnut branches.

Vulnerability curves show the mean percentage loss of xylem conductivity (*PLC*, %) plotted versus the xylem water potential. Lines are logistic fitted to the data and error bars are SE (n = 3 to 6). *(A)*, xylem vulnerability to cavitation was measured on 28-cm-long segments (■) and 38-cm-long segments (□). *(B)*, xylem vulnerability curves obtained when water flows from the bottom to up of the sample (●, sense flow) or from up to bottom of the sample (○, antisense flow). Normal sigmoidal curves were obtained from both senses of water flow; however, the antisense flow gave data that fitted better to the logistic line (R^2 = 0.979 and 0.994 for sense and antisense flows, respectively).

Where V_s is the fresh volume (cm^3) and M_0 is the dry mass (g).

Fresh volumes of three mm long segments were measured according to the principle of Archimedes by water displacement on an analytical balance. In order to gain the fresh volume as accurately as possible, sample segments were previously fully hydrated by placing them in deionised water under vacuum. Thereafter, samples were dried in drying kiln at 70˚C for 2 days and their dry weights were measured.

For wood anatomy analyses, cross sections of the middle part of the samples were prepared and dyed with Safranin. They were observed under an optical microscope (X 20) and images were recorded by a digital camera (AxioCam HR, Zeiss). Wood anatomy was deciphered by image analysis using ImageJ software (Rasband, 1997-2009). The vessels were isolated by automatic segmentation then their diameters (D, µm), density (VD, number mm^{-2}) and the lumen vessel area (A_v, %) were measured.

7. Statistics

The analysis of variance (ANOVA) was used to test the effects of the following parameters on the vulnerability to cavitation: length of branch segments, sense of water flows and walnut cultivars. When significant differences were found, Duncan's multiple range test with P-value < 0.05 was carried out.

Results

1. Methodological analysis on the measurement of vulnerability to cavitation

The VCs obtained with 0.28 and 0.38 m long segments appeared to have slight differences in shape, and their respective mean P_{50} were significantly different ($P < 0.05$) with values of −1.65 MPa and −1.99 MPa respectively (Figure 1*A*). Air bubbles were observed on some branch segments of 0.28 m long while none was observed on 0.38 m long branches, when infiltrated them with air. These results indicated that maximum vessel length of the walnut cultivars studied was shorter than 0.38 m and can be longer than 0.28m. We tested both water flow directions through sample while spinning on the Cavitron (Figure 1*B*). VCs from both sense and antisense flows were sigmoidal and did not show significant difference in P_{50} (−1.99 and −2.02 MPa, respectively). However, data set from antisense flow fitted better to the logistic line than data set from sense flow. The coefficient of determination (R^2) for sense

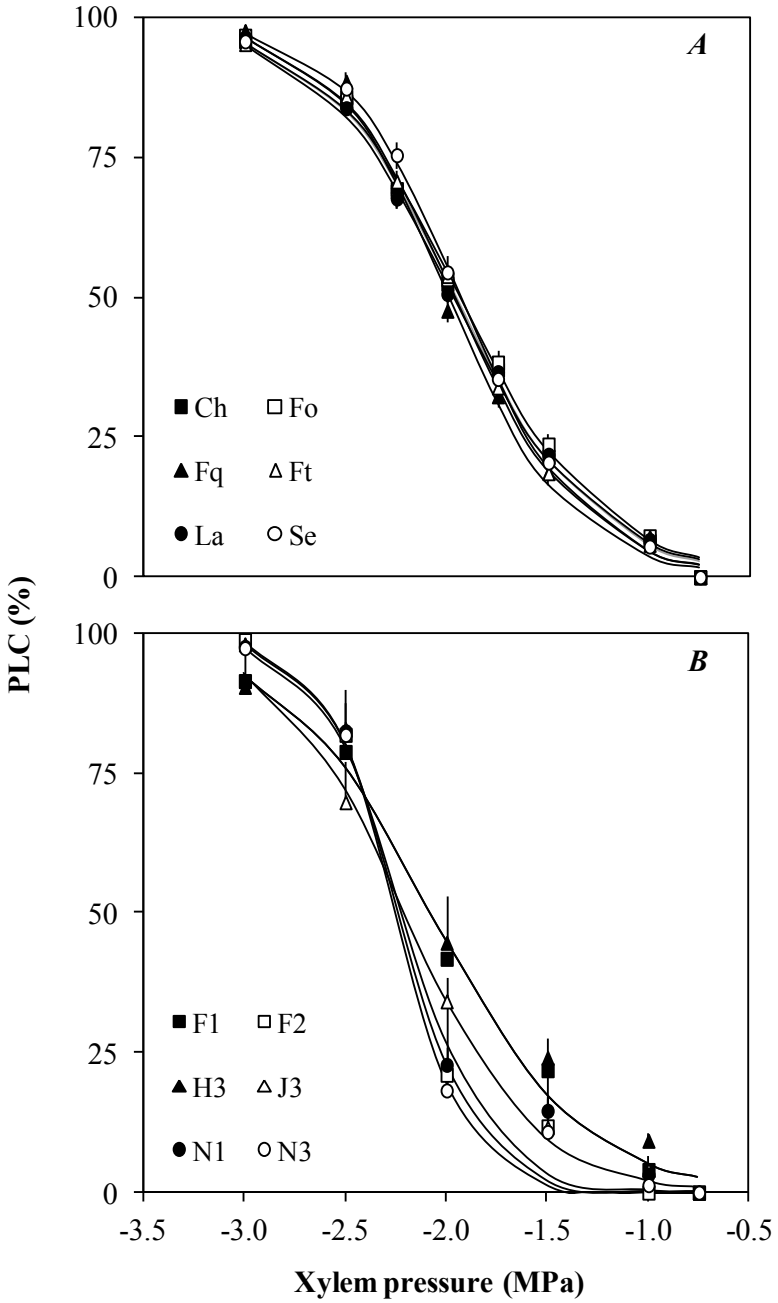

Figure 2. Xylem vulnerability to cavitation of Persian walnut cultivars (*A*) and hybrid walnut cultivar (*B*).

Vulnerability curves show the percentage loss of xylem conductivity (*PLC*, %) plotted versus the xylem water potential. Measurements were conducted on (*A*) six cultivars of *J. regia*: Chandlers (Ch, n = 15), Fernettes (Ft, n = 13), Fernors (Fo, n = 10), Franquettes (Fq, n = 67), Laras (La, n = 42), and Serrs (Se, n = 18) and (*B*) six hybrids of *J. regia* x *J. nigra*: F1, F2, H3, J3, N1 and N3 (n = 4). Dots are mean values with fitted logistic lines and bars are *SE*.

and antisense flows were 0.979 and 0.994 respectively. We thus continued the analyses with 0.38 m long samples and antisense flow.

2. Xylem vulnerability to cavitation on Persian and hybrid walnuts

Values of *LMA* of the six Persian walnut cultivars ranged from 108.8 to 114.7 $g.m^{-2}$ and did not vary significantly ($P > 0.05$). These results ensured that branch samples were subjected to the same environmental conditions, and that there were no different light effects on the studied traits between the cultivars studied. This was an important test since light conditions were known to affect the *VC* (Cochard *et al.*, 1999; Herbette *et al.*, 2010).

Native embolism measured on Persian walnuts ranged from 22.71 to 38.41% ($\bar{\chi}$ = 30.70 ± 5.82%) while on hybrids it ranged from 13.80 to 27.14% ($\bar{\chi}$ = 19.09 ± 7.08%), neither did show significant differences between mean values in native embolism ($P < 0.05$). This test was a prerequisite in order to avoid the effect of the native embolism on the vulnerability to cavitation measured with Cavitron.

Vulnerability curves are shown in Figure 2 (*A)* for 6 Persian walnut cultivars and (*B)* for 6 hybrid walnuts. The vulnerability to cavitation parameters (P_{12}, P_{50}, P_{88} and s) obtained for the two species tested are presented in Table 1. Both the vulnerability curves and P_{50} values were not significantly different among Persian walnut cultivars, mean P_{50} ranking from −1.93 to −1.98 MPa. Most of variability was observed within cultivar (67.28%) while the other sources of variation in P_{50} were attributed to tree-cultivar interaction (15.36%), tree effect (15.04) while only 2.32% was the effect of cultivars. Weak but significant differences were found for P_{12}, P_{88} and s parameters between cultivars. No significant differences were found for xylem vulnerability to cavitation parameters ($P > 0.05$) among hybrid walnut tested. For P_{50}, values ranked from −2.05 to −2.23 MPa, the variation was mostly observed within hybrids (78.35%). When compared 2 walnut species tested, significant differences were found for all vulnerability to cavitation parameters (Table 1 and 2, $P < 0.05$). Hybrids walnuts appeared to be less vulnerable to cavitation than Persian walnuts (P_{50} = −2.16 and −1.97 MPa respectively); 80.96% of variance was found between the provenances while the rest of variation was contributed by variation between cultivars (19.02%). Nevertheless, for P_{88} and s parameters, the variation was found mostly between cultivars (66.02 and 66.70%

Table 1. Parameters of xylem vulnerability to cavitation in walnut cultivars.

	Cultivars	P_{12} (MPa)	P_{50} (MPa)	P_{88} (MPa)	s (%/MPa)
J. regia	Chandler (Ch)	−1.29 (0.04) ^{a}	−1.95 (0.03) ^{a}	−2.61 (0.04) ^{ab}	78.04 (3.77) ^{bc}
	Fernor (Fo)	−1.25 (0.04) ^{a}	−1.94 (0.03) ^{a}	−2.62 (0.03) ^{ab}	73.98 (2.57) ^{c}
	Franquette (Fq)	−1.40 (0.02) ^{b}	−1.98 (0.01) ^{a}	−2.56 (0.02) ^{a}	89.46 (2.20) ^{a}
	Fernette (Ft)	−1.33 (0.04) ^{ab}	−1.96 (0.02) ^{a}	−2.58 (0.03) ^{ab}	81.68 (3.81) ^{abc}
	Lara (La)	−1.29 (0.02) ^{a}	−1.97 (0.02) ^{a}	−2.65 (0.03) ^{b}	76.14 (2.29) ^{c}
	Serr (Se)	−1.34 (0.03) ^{ab}	−1.93 (0.02) ^{a}	−2.52 (0.04) ^{a}	88.84 (4.65) ^{ab}
	Mean	**−1.34 (0.01) ^{A}**	**−1.97 (0.01) ^{A}**	**−2.59 (0.01) ^{A}**	**83.41 (1.37) ^{B}**
J. regia x *J.nigra*	F1	−1.34 (0.07) ^{a}	−2.07 (0.04) ^{a}	−2.80 (0.03) ^{a}	68.77 (3.51) ^{a}
	F2	−1.84 (0.08) ^{a}	−2.23 (0.06) ^{a}	−2.62 (0.10) ^{a}	143.38 (32.41) ^{a}
	H3	−1.35 (0.19) ^{a}	−2.05 (0.08) ^{a}	−2.75 (0.23) ^{a}	88.46 (22.99) ^{a}
	J3	−1.58 (0.11) ^{a}	−2.20 (0.08) ^{a}	−2.82 (0.07) ^{a}	82.02 (6.28) ^{a}
	N1	−1.81 (0.27) ^{a}	−2.20 (0.13) ^{a}	−2.59 (0.02) ^{a}	230.24 (114.54) ^{a}
	N3	−1.84 (0.16) ^{a}	−2.23 (0.08) ^{a}	−2.62 (0.06) ^{a}	149.31 (2.95) ^{a}
	Mean	**−1.63 (0.07) ^{B}**	**−2.16 (0.03) ^{B}**	**−2.70 (0.04) ^{B}**	**127.03 (21.81) ^{A}**

Xylem pressure causing 12, 50 and 88% loss of xylem hydraulic conductivity (P_{12}, P_{50} and P_{88}) and the slope of the vulnerability curve (s) were calculated from the curves of vulnerability to cavitation in 6 cultivars of *J. regia* and in 6 hybrids of *J. regia* x *J. nigra*. Values represent means with standard error in the brackets, n = 4 for each hybrids while n ≥ 10 for each *J. regia* cultivars. Means were compared between *J. regia* cultivars, between hybrids and between two species. Values with different letters are significantly different at $P < 0.05$ (Duncan's multiple range test).

respectively) while for P_{12} the variation was equally distributed between provenances and between cultivars (Table 2).

3. Wood density and wood anatomical traits

The means of *WD* for 6 studied Persian walnut cultivars varied from 0.39 to 0.45 g.cm^{-3} with significant differences ($P < 0.05$) between Se and the following cultivars; Fo, Fq, Ft and La while it was similar to Ch (Table 3). Statistical analysis indicated no significant difference ($P > 0.05$) between the six cultivars for the mean values of *D* and *VD*. However, significant difference ($P < 0.05$) was found for A_v (Table 3) between the cultivars Ch and Se showing the highest A_v and the cultivar 'Ft' showing the lowest A_v.

Discussion

The investigation on genetic variability in xylem vulnerability to cavitation is of interest for breeding program in drought tolerance. For Persian walnut production, the importance of exploring this trait is highlighted by the location of this crop species in drought prone areas. It may help breeders to select for more suitable genotypes/ cultivars which will survive in unfavorably dry conditions with less irrigation inputs for the coming future.

The centrifugal technique 'Cavitron' (Cochard, 2002; Cochard *et al.*, 2005) is an efficient and rapid method for large scale investigations on xylem vulnerability to cavitation (Corcuera *et al.*, 2011; Lamy *et al.*, 2011; Wortemann *et al.*, 2011). Nevertheless, this method can only be applied to species having short conduits, since conduits that are longer than the sample length generate a measurement artifact and an abnormal shape of the *VC* (Cochard *et al.*, 2010; Martin-StPaul *et al.*, 2014). The results on maximum vessel length measured on Persian walnuts indicated that 0.38 m long segments, but not 0.28 m long ones, were longer than maximum vessel length, and resulted in a sigmoid *VC*. From these results, we recommend using 0.38 m long segments for the building of *VC* of walnut tree. We also recommend working with the antisense flow direction since it gave better-fit to logistic line data when compared to the sense flow. A slightly faster increase in embolism rate in the latter case would be due to higher number of cut open vessels at the proximal end that embolized quickly when nuclei are seeded into them by measurement flows.

Native embolism can lead to an underestimation of xylem vulnerability to cavitation when only few conduits are still functional during measurements (usually the most resistant

Table 2. Correlations of xylem vulnerability to cavitation parameters between Persian and hybrid walnuts.

	P_{12} (MPa)		P_{50} (MPa)		P_{88} (MPa)		s (%/MPa)	
	R^2	P	R^2	P	R^2	P	R^2	P
Between species	0.50	**< 0.0001**	0.81	**< 0.0001**	0.34	**0.002**	0.33	**< 0.0001**
Between cultivars	0.50	**< 0.0001**	0.19	0.330	0.66	0.075	0.67	**< 0.0001**

Values indicate correlation of determinations (R^2) and P values (P). Significant correlations are shown with bold letters ($P < 0.05$).

Table 3. Wood anatomical traits of 6 Persian walnut cultivars.

Cultivar	Chandler (Ch)	Fernor (Fo)	Franquette (Fq)	Fernette (Ft)	Lara (La)	Serr (Se)
WD (g.cm^{-3})	0.42 (0.01) ^{ab}	0.45 (0.02) ^{a}	0.45 (0.01) ^{a}	0.43 (0.01) ^{a}	0.43 (0.01) ^{a}	0.39 (0.01) ^{b}
D (μm)	75.46 (4.48) ^{a}	72.09 (2.13) ^{a}	76.80 (2.31) ^{a}	75.07 (3.09) ^{a}	81.46 (6.03) ^{a}	80.68 (4.39) ^{a}
VD (number. mm^{-2})	42.56 (5.69) ^{a}	44.16 (4.61) ^{a}	35.32 (4.40) ^{a}	41.32 (2.91) ^{a}	31.89 (5.55) ^{a}	46.26 (3.50) ^{a}
A_v (%)	0.27 (0.02) ^{a}	0.19 (0.01) ^{ab}	0.20 (0.01) ^{ab}	0.17 (0.01) ^{b}	0.21 (0.03) ^{ab}	0.27 (0.03) ^{a}

Wood density (WD) was measured on Chandlers (Ch, n = 15), Fernettes (Ft, n = 13), Fernors (Fo, n = 10), Franquettes (Fq, n = 68), Laras (La, n = 42), and Serrs (Se, n = 18) while vessel diameter (D), vessel density (VD) and vessel lumen area (A_v) were analyzed on five branches for each cultivar. Values represent means with standard error in the brackets. Values with different letters are significantly different at $P < 0.05$ (Duncan's multiple range test).

conduits to cavitation). The rather low native embolism we measured here ensured that xylems of the samples were not greatly embolized by previous drought or frozen stresses. This indicates that xylem vulnerability to cavitation was measured on large proportion of functional conduits avoiding any bias.

The P_{50} values obtained in this study (means of P_{50} = −1.95 and −2.16 MPa for Persian and hybrid walnuts respectively) were in accordance with the previous results obtained from *J. regia* L. cv Lara (Tyree *et al.*, 1993) and *J. nigra x J. regia* cv NG38 (Cochard *et al.*, 2002) with different analytical techniques. The P_{50} values for the current year branches from these previous studies were −2.10 MPa. Similar xylem vulnerability to cavitation found for the six cultivars of Persian walnuts is rather surprising since differences in drought resistance traits were observed from previous studies between cultivars. For example, differences in $\delta^{13}C$ were found between 22 Persian walnuts from different provenance regions, indicating a variation in water use efficiency (Aletà *et al.*, 2009). The cultivars from drought-prone provenance regions were found more efficient in water use. This assumption arises from a great vulnerability to cavitation that was highlighted when comparing *Prunus* species (Cochard *et al.*, 2008). Despite significant differences found for some of wood anatomical traits between cultivars of Persian walnut, the values were remarkably close. These results may contribute to similar vulnerability to cavitation found among the Persian walnuts found in this study since previous studies have demonstrated correlation between these two traits (Baas *et al.*, 2004; Hacke *et al*., 2001).

Because of the lack of difference between these Persian walnut cultivars, we decided investigating the vulnerability to cavitation in *J. regia* x *J. nigra* hybrids in order to enhance the range in genetic diversity. However, we also found similar vulnerability to cavitation among hybrid walnuts studied, only weak significant differences were found when compared this trait between the species. These similar/slightly differences in cavitation resistance suggesting ‘uniform selection’ on this trait in walnut trees hence canalizing cavitation resistance of the studied walnuts. This finding is consistent with the results of previous studies on *Pinus* (Lamy *et al.*, 2011; Lamy *et al.*, 2014) which indicating no genetic variability in cavitation resistance among *Pinus* populations. Altogether these results suggested that vulnerability to cavitation does not vary in branch of walnut tree and that differences in drought resistance rely rather on drought avoidance traits, segmentation in xylem vulnerability to cavitation between branch and petiole was reported in walnut tree (Tyree *et*

al., 1993). A variation in cavitation resistance of petiole could thus be expected for petiole in walnut cultivars.

Differences between cultivars and hybrids were found for various traits such as the budburst date, the heat requirement and the growth rate (Charrier *et al.*, 2011; Poirier *et al.*, 2004), despite small difference in cavitation resistance. It is thus possible to carry out selection program for other interested traits without effect on xylem vulnerability to cavitation. In addition, a rather low cavitation resistance for these commercial cultivars indicates a great potential for breeding program to improve on this trait. The challenge will be to find genotypes that are more resistance to cavitation such as populations from extremely dry locations.

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II. Clonal variability for vulnerability to cavitation and other drought-related traits in *Hevea brasiliensis* Müll. Arg.

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Acknowledgement

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Clonal variability for vulnerability to cavitation and other drought-related traits in *Hevea brasiliensis* Müll. Arg.

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Abstract

Selection for drought-tolerant clones has become a major challenge in rubber breeding programs undertaken to ensure the sustainability of natural rubber production, as rubber plantations are expanding in drought-prone areas. Xylem vulnerability to cavitation is a trait related to drought-induced mortality. It can be rapidly evaluated without subjecting plant materials to drought stress, making it useful in large-scale screening for drought tolerance in the near future. We first compared the most widely used techniques for measuring vulnerability to cavitation (air pressurization and Cavitron) on this species, and the effect of sample conditions (size, age and sunlight exposure), in order to ensure reliable analysis. Secondly, ten rubber clones were compared for their xylem vulnerability to cavitation in branches and petioles, and for other traits related to drought response, including stomatal response and leaf shedding occurring during a simulated drought. We also tested the plasticity of vulnerability to cavitation on two clones grown in three locations with contrasting precipitation regimes. We found no clonal variability and a small phenotypic plasticity for xylem vulnerability to cavitation in branches. However, clonal differences in xylem vulnerability to cavitation were found in petioles, and clones also showed differences in stomatal response and in leaf shedding behavior in response to a simulated drought. Our study suggests a genetic canalization for vulnerability to cavitation in organs critical for survival, such as branches, whereas there are clonal differences for traits related to drought avoidance: vulnerability to cavitation of petioles, leaf shedding behavior and stomatal response. The insights gained in this study for screening rubber tree clones for drought tolerance is also discussed.

Keywords: cavitation, clonal variability, drought, embolism, *Hevea brasiliensis*, hydraulics, leaf shedding, stomatal conductance, xylem.

Introduction

The rubber tree (*Hevea brasiliensis* Muell. Arg.) is an important agricultural species. It is native to the Amazonian basin, and extensively planted in Southeast Asia. In Thailand, it was originally cultivated in the southern and eastern provinces where rainfall is abundant. However, owing to shortages of land for cultivation and competition with other crops in these regions, plantations have spread northward into northern and northeastern provinces (Fox and Castella, 2013). These areas are considered marginal for the cultivation of rubber tree in view of rainfall distribution and water availability due to poor soil

conditions (Boithias *et al.*, 2012). Yet in 2011, the area of rubber plantations in these marginal regions increased by 12% compared with 2006 (RRIT, 2011). Drought stress has been reported to impair the growth of rubber trees (Chandrashekar *et al.*, 1998; Manmuen *et al.*, 1993) and latex production (Wichichonchai and Manmeun, 1992). Thus the northward expansion of rubber plantations can bring natural rubber production under a higher risk of drought stress. This is a real threat because extreme drought events are predicted in the years to come (Chinvanno, 2011; IPCC, 2013). Also, the use of a single rubber clone (RRIM600) in over 80% of the plantation area in Thailand further increases this risk. In this context, the selection for drought-tolerant clones has become a major challenge for rubber breeding programs to ensure yield and minimize additional costs for water management in rubber production.

Several strategies have been proposed for the selection of rubber clones suitable for cultivation in marginal areas, such as the evaluation of prominent clones and polyclonal seedlings, recombination breeding and selection, and the evaluation of Brazilian germplasm (Priyadarshan and Goncalves, 2003). The selection of rubber tree is time-consuming because it is strongly limited by non-synchronous flowering, low fruit setting, a long juvenile period, and the heterozygous nature of rubber trees (Venkatachalam *et al.*, 2007). Hence there is a need for early screening tests for drought tolerance to speed up the process of progeny testing and produce genetic material adapted to the climatic conditions in the coming decades. Previous studies have shown high polymorphism in wild and cultivated rubber populations (Besse *et al.*, 1994; Lekawipat *et al.*, 2003), differences in growth (Chandrashekar *et al.*, 1998) and yield potential (Priyadarshan *et al.*, 2005) in rubber clones from several sub-optimal areas. Variability in drought tolerance can thus be expected in rubber clones.

Xylem sap is normally transported under tension (Dixon and Joly, 1895), which puts xylem sap in a metastable state. Under drought, this tension is exacerbated and the xylem sap can rapidly change into vapor (embolism) and cause cavitation of the xylem (Tyree and Sperry, 1989). This drought-induced cavitation may lead to a reduction in water transport and cause organ or plant death (Barigah *et al.*, 2013). Xylem vulnerability to cavitation is considered an important trait determining drought tolerance in woody species (Choat *et al.*, 2012). It ranges widely across the species and correlates with drought tolerance of the species (Maherali *et al.*, 2004; Pockman and Sperry, 2000; Tyree *et al.*, 2003). Within-species variation of xylem vulnerability to cavitation has been observed in several forest species; the diversity may be due to genetic control (Cochard *et al.*, 2007; Wortemann *et al.*, 2011) or a result of plant adjustment to the environmental conditions (Awad *et al.*, 2010; Corcuera *et al.*, 2011; Herbette *et al.*, 2010). The xylem vulnerability to cavitation also varies between plant organs (Tyree *et al.*, 1993), indicating vulnerability segmentation, which allows the plant to discard expendable organs in order to preserve critical ones (Zimmermann, 1983). Small genetic differences are observed between natural populations of some forest species (Lamy *et al.*, 2011; Wortemann *et al.*, 2011), which is interpreted as a genetic canalization for this trait, i.e. a reduction in phenotypic variability regardless of genetic variability. For agricultural species, the genetic variation of xylem vulnerability to cavitation remains to be tested, as these have undergone selection pressure different from natural species (Gepts, 2004; Purugganan and Fuller, 2009).

Various techniques have been developed to estimate xylem vulnerability to cavitation; the recent and most rapid techniques include Cavitron centrifugation (Cochard *et al.*, 2005) and air-injection (Ennajeh *et al.*, 2011). These methods allow the estimation of xylem vulnerability to cavitation on plant segments without subjecting plants to drought stress, and so significantly reduce experimentation time. However, there is an ongoing debate on the reliability of these techniques especially with long-vessel species (Choat *et al.*, 2010; Cochard *et al.*, 2010; Cochard *et al.*, 2013; Delzon and Cochard, 2014; Ennajeh *et al.*, 2011; Jacobsen and Pratt, 2012; Sperry *et al.*, 2012; Tobin *et al.*, 2013; Torres-Ruiz *et al.*, 2014).

In this study, we firstly investigated genetic variability in xylem vulnerability to cavitation in ten commercialized rubber clones used in Thailand, and looked at the environmental plasticity of this trait in two clones from three locations that differ in mean annual precipitation. Secondly, we compared the responses of the clones to drought stress. Before investigating vulnerability to cavitation, we tested the reliability of two techniques, Cavitron and air-injection, on rubber tree branches, and the effects of plant material properties (sample length, sunlight exposure and age) on the vulnerability curves. This is a prerequisite to choosing the most suitable and accurate method to assess xylem vulnerability to cavitation in *H. brasiliensis*.

Materials and Methods

Plant materials

The studies were conducted using plant materials from three different locations in Thailand: *i*), Khon Kaen (16°48'21"N, 103°08'22"E; altitude 252 m), (*ii*) Nong Khai (18°09'30"N, 103°09'31"E; altitude 171 m), and (*iii*) Surat Thani (9°40'27"N, 99°6'40"E; altitude 19 m). The first two locations are situated in the northeastern region of Thailand whereas the latter location is in the

southern region. They had contrasting average annual rainfall and dry season length; defined as the number of months with less than 50 mm of rain (Webster and Baulkwill, 1989). At Surat Thani, the rainfall regime is considered non-limiting for rubber trees, with an annual rainfall of 1,800 mm and dry conditions of only one to two months per year. The annual rainfall of Khon Kaen and Nong Khai are 1,200 mm and 1,600 mm, respectively with a dry season of five to six months (Thai Meteorological Department, 2014). These two locations are considered to be in a marginal area for natural rubber production, because rubber trees require more than 1,250 mm of annual rainfall with 120–150 rainy days per year for optimal growth and production (RRIT, 2012).

The experiment can be divided into two main studies: the investigation of the variation of xylem vulnerability to cavitation and the analysis of plant response to drought stress.

For the investigation of the variation of xylem vulnerability to cavitation, we firstly investigated the reliability of the techniques and the effects of sample properties on three commercial clones (PB 235, RRIM 600 and RRIT 251) grown at Surat Thani site. Branches were collected on April 2012 from three to four individual trees for each test and each condition. Table 1 describes the samples used for comparing analytical methods (Cavitron versus air-pressure) and for testing effects of sample ages (current year and previous year branches), sunlight exposure (shaded and sun-exposed) and sample size. Then after, genetic diversity and environmental plasticity of xylem vulnerability to cavitation were investigated. The study of genetic variability was conducted on ten commercial rubber clones: BPM 24, PB 217, PB 235, PB 260, PB 5/51, RRII 105, RRII 118, RRIM 600, RRIT 251 and RRIT 408. Clones (n = 12–15 trees/clone) were grafted on RRIM 600 rootstocks and planted in the field at Nong Khai site in October 2012. On March, 2013, current year branches (n = 6–8/clone) were harvested from these trees. The study of environmental plasticity was carried out using branches of two clones (RRIM 600 and RRIT 251) from all three indicated sites. At each location, ten current year branches from the fully sun-exposed side of the canopy were harvested for each clone from one- to two-year old trees on November 2013.

In the study of plant response to drought stress, the stress was carried out on the same ten rubber tree clones used for investigating the genetic variability of xylem vulnerability to cavitation. The trees used in this experiment were also grafted on RRIM 600 rootstocks, but were individually planted in 0.93 m^2 pots filled with sandy loam soil at Nong Khai site on October 2012. Each clone consisted of six replicates later divided into two groups of watering regimes: control and stress groups. During the growing period, each tree was watered to full field capacity with 15 liters twice a week until December 7 2012. Drought stress was then applied to the trees in the stress group by withholding the water supply, leaving the soil to dry down. The control trees were kept growing under full field capacity. Drought stress lasted for 11 weeks until high level of xylem embolism in petioles was reached, and on March 20 2013 watering was resumed for the stressed trees. During the drought experiment, occasional rains were strictly controlled by portable covering systems. They were installed to cover the pots before the rain came on, and removed promptly afterward to prevent the temperature rising in the pots.

Methods

The study of xylem vulnerability to cavitation

After severing, branch segments 0.60 m long were immediately defoliated and prepared for shipping to France for the analysis of xylem vulnerability to cavitation. The defoliated leaves from branches used for the study of genetic variability (n = 40/clone) were collected and measured for leaf mass per unit area (LMA). Branches were prepared for the shipping to France for the analysis of xylem vulnerability to cavitation by covering their ends with paraffin, treating them with pesticide, and sealing them in transparent plastic bags. These bags were then packed in cartons with packaging buffer and sent to France by air in ambient temperature. It took approximately one week for the package to arrive in France. On arrival, native embolism was measured on six randomly chosen branches. This is a prerequisite measurement proposed and explained by Awad *et al.* (2010). The remaining branches were rewrapped with moist paper, placed in a plastic bag and carton, and stored at 4°C. Branches were brought out of cool storage just long enough for a daily analysis of xylem vulnerability to cavitation by Cavitron or air-injection. These procedures are considered a standard protocol for the sampling and sample preparation, and were applied for all other branch samplings for xylem vulnerability to cavitation measurement in this study.

Native embolism

When branches arrived at the laboratory in France, native embolism was immediately measured. For the samples designated for tests of techniques and sample properties, six branches were randomly chosen for this measurement. For the study of genetic variability and environmental plasticity, 20 (n = 2/clone) and 18 branches (n = 3/clone/location) were randomly chosen. The remaining branches were rewrapped with moist paper and stored at 4 °C. The maximum storage time before branches were measured for the vulnerability to cavitation for three experiments was 7, 10 and 30 days.

Native embolism was measured with Xyl'EM (Bronkhorst, France) following the procedure described by Cruiziat *et al.* (2002). A segment 0.10 m long was harvested from the central part of the branches; it was cut under water to prevent air from entering xylem conduits and causing artificial embolism. The proximal end of the segment was attached to the hydraulic apparatus, and a solution containing 10 mM KCl and 1 mM $CaCl_2$ was perfused through the segment. Initial hydraulic conductance (K_i) and maximum hydraulic conductance (K_{max}) were then measured successively under low pressure (6–9 kPa). Before K_{max} was measured, the emboli were removed by applying a series of 3 min flushes (0.15 MPa) with the 10 mM KCl and 1 mM $CaCl_2$ solution. The percentage loss of xylem conductivity (PLC) was calculated by:

$$PLC = 100 \times (1 - \frac{K_i}{K_{max}}) \quad (1)$$

Branch xylem vulnerability to cavitation

The measurement of xylem vulnerability to cavitation on rubber branches was made using the Cavitron centrifuge technique (Cochard, 2002; Cochard *et al.*, 2005) and the 'pressure sleeves' air-injection technique (Ennajeh *et al.*, 2011). All the investigations on xylem vulnerability to cavitation in branches used the Cavitron technique. The analysis of genetic variability to cavitation in ten rubber clones was measured on branch segments 0.37 m long. The analysis of environmental plasticity was carried out with segments 0.41 m long. The small difference in sample size between the two experiments was due to a change in the rotors of the Cavitron we used, which differed in diameter (0.38 and 0.42 cm, respectively).

Branches were peeled to remove the bark containing the laticifer and so prevent latex exudation that could block the xylem vessels during the measurement. They were successively cut to the desired length, and branches were then infiltrated with compressed air (0.10 MPa) at one end of the segment while the other end was submerged in water. This was done to check for vessels open at both ends in the segments (Ewers and Fisher, 1989; Zimmermann and Jeje, 1981). If vessels are open at both ends, air bubbles are observed at the submerged end. Only branch segments with no open vessels were used for the analysis of vulnerability to cavitation. This was to exclude the reported effect of the open vessel artifact on the vulnerability curve when using Cavitron (Cochard *et al.*, 2013; Delzon and Cochard, 2014; Torres-Ruiz *et al.*, 2014).

The Cavitron technique uses centrifugal force to increase the tension in vessels, while measuring the decrease in hydraulic conductance. Xylem pressure (P) was set to a reference pressure (−0.75 MPa) and the K_{max} value was first measured. The tension was then increased by either −0.25 or −0.50 MPa, and new sample conductance (K_i) was measured. PLC was computed using Equation (1). These procedures were repeated until sample PLC reached more than 80%. The vulnerability curve (VC) was then constructed by plotting PLC versus P and fitting with a sigmoid function (Pammenter and Van der Willigen, 1998):

$$PLC = \frac{100}{1 + e^{(\frac{s}{25 \times (P - P_{50})})}} \quad (2)$$

The pressure inducing 50% loss of xylem conductivity (P_{50}, MPa) and the slope of the VC (s) were determined from the fitted sigmoid curve. The xylem tension causing 12% and 88% loss of xylem conductivity (P_{12} and P_{88}, respectively) were calculated as:

$$P_{12} = P_{50} + \frac{50}{s}, \quad (3)$$

$$P_{88} = P_{50} - \frac{50}{s}. \quad (4)$$

P_{12} is considered as the 'air entry point' in which the embolism begins (Sparks and Black, 1999; P_{88} is the tension before xylem becomes totally non-conductive or 'full embolism point' (Domec, 2001).

For the air-injection technique, a pressure sleeve was made up of three-way steel tubes from Swagelok Company (2.50 cm in diameter and 7.50 cm long). It was applied to the middle of a branch segment 0.37 m long. Bark was peeled off at the center of the branch to create a contact surface (3.50 cm long) with compressed air inside the chamber. This surface allows the air to enter the xylem and induces xylem embolism (Ennajeh *et al.*, 2011). The distal end of the branch segment was attached to a vertical plastic tube filled with a solution of 10 mM KCl and 1 mM $CaCl_2$, while a plastic tube filled with cotton was placed at the proximal end to collect the solution flowing through the sample. Embolism was induced by increasing air pressure in the chamber sleeves. The hydraulic conductance (K) was calculated as:

$$K = \frac{F}{P}, \quad (5)$$

where F is the flow rate through sample calculated as the weight of water collected for 1 min, and P is the solution pressure in the vertical plastic tube connected to the sample. PLC was calculated using Equation (1). Air pressure in the chamber was increased stepwise until more than 90% PLC was achieved. The VC was constructed by plotting PLC versus P and then fitting with sigmoid curve using Equation (2). The characteristics of the xylem vulnerability to cavitation (P_{12}, P_{50}, P_{88} and s) were calculated.

Petiole vulnerability to cavitation

Native embolism was measured on the field in petioles bearing the leaflets used for the Ψ_{md} measurements, in all trees. Measurements were performed on a segment

0.05 m long harvested from the central part of the petiole segment as described above for branches. Petiole vulnerability to cavitation was determined by plotting the native embolism values versus Ψ_{md} measured on the same leaves, as drought stress progressed. Data was fitted with the sigmoid function following Equation (2), P_{50} and s were extracted from the fitted curve and P_{12} and P_{88} were later calculated using Equations (3) and (4), respectively.

The study on plant response to drought stress

During the dry-down experiment, changes in traits of interest (soil water content, leaf water traits, and leaf and growth traits) were measured.

Soil water content

To follow the progression of drought stress, 101.38 cm^3 of soil was collected at depth 0.30 m and 0.60 m from each pot. The soil was weighed and oven-dried at 105 °C for 24 hours, and its dry weight was measured. Soil water content (SWC) was calculated as: SWC = 100 × (fresh weight − dry weight) / dry weight. The sampled soil was returned to the pots after each measurement of SWC to maintain soil volume in the pot.

Leaf water traits

The minimum stomatal conductance (g_s, mmol m^{-2} s^{-1}) and the midday leaf water potential (Ψ_{md}, MPa), were measured one to two times a month with a porometer (AP4, Delta-T Device, Cambridge, U.K.) and a pressure chamber (plant water status console model 3005F1, Soilmoisture Equipment Corp., CA.), respectively. These two traits were measured between 12:00 and 14:00 under full sunlight conditions. The value of g_s was measured on three leaflets from three individual compound leaves situated at the lowest level of leaf flush on each tree. We made sure that the measurements were conducted on the leaves located at a relatively similar position on every tree. When there was no more leaf at the lowest level on a tree, the measurements were moved up to a higher position for all trees. The relative stomatal conductance (g_s /$g_{s\ max}$, %) was calculated: g_s was the minimum stomatal conductance from stressed trees, and $g_{s\ max}$ was the minimum stomatal conductance averaged from the control trees, both g_s and $g_{s\ max}$ being measured on the same day. Following the g_s measurement, one of the compound leaves on a tree was chosen; it was covered with a plastic bag along with its petiole and removed for the measurement of Ψ_{md} and PLC. A segment of petiole at least 0.10 m long was cut and kept under tap water for measurement of the petiole PLC as described below. The remainder the leaf in the plastic bag was immediately placed in a pressure chamber for the Ψ_{md} measurements.

We plotted gs /gs max versus Ψmd and fitted these data with the sigmoid function according to Equation (2) to calculate the water potential causing 50% of stomatal closure (gs 50). Water potentials causing 12% and 88% of stomatal closure (gs 12 and gs 88, respectively) were then calculated using Equations (3) and (4).

Leaf and growth traits

For each clone, leaf area was measured on 40 leaves using a leaf area meter (LI-3000A, LI-COR Inc.), and a mean leaf size was calculated. Ten of these leaves were then used to measure leaf mass per unit area (LMA). Their dry mass was measured after drying them at 70 °C for 48 hours in a gravity convection oven. The LMA was calculated as the ratio of leaf dry weight to leaf area.

During the experiment, the number of leaves and the tree height were recorded every four weeks. The total leaf area of the tree was estimated based on the number of leaves, and the leaf size averaged for each clone. Accumulated shed leaf area was determined for each tree, and the relative shed leaf area (LS) was calculated. The LS was expressed as the drought-induced leaf shedding compared with leaf shedding observed on control trees. LS was then plotted against Ψ_{md} and fitted with the sigmoid function according to Equation (2). We then calculated the xylem tension (MPa) that caused 12%, 50% and 88% of leaf shedding (LS_{12}, LS_{50} and LS_{88}, respectively). The growth rate (GR) was calculated from the slope of the logarithm of height *versus* time over the 11 weeks of drought stress.

Hydraulic safety margins

The hydraulic safety margin of a plant can be determined using various traits involving the threshold tensions for safety and efficiency in xylem conduits (Choat *et al.*, 2012; Markesteijn *et al.*, 2011; Meinzer *et al.*, 2009). In this study, we determined the hydraulic safety margins for petiole and branch according to the following hypotheses: (*i*) stomatal regulation prevents petiole xylem from reaching a threshold tension inducing cavitation, and (*ii*) drought-induced leaf shedding helps preserve the branch from embolism. The petiole safety margin was calculated as the difference between the xylem pressure at $g_{s\ 50}$ and the P_{50} of the petiole. The safety margin of the branch was calculated as the difference between LS_{50} and the P_{50} of the branch.

Statistics

Analysis of variance (ANOVA) was used to test the effects of the following factors on the xylem vulnerability to cavitation: techniques (Cavitron versus air-injection), lengths of branch segment (0.27 m, 0.37 m and 0.41 m), light exposures (shaded and fully exposed), sample ages (current and previous year

branches), clones and water availability in plantations. ANOVA was also used to test the effect of water deficit treatment on the following traits: SWC, Ψ_{md}, g_s /$g_{s\ max}$, PLC, LS, safety margin of the branch and petiole, and GR. When significant differences were found, we performed the Tukey-Kramer multiple comparison at 95% confidence interval.

Results

Reliability of Cavitron and air-injection techniques and the effects of sample properties

Native embolism measured on three sets of branches indicated a rather low native embolism with a mean of 15.61% (SE ± 1.54). No significant difference was found either within or between sampling sets. When the segments were infiltrated with compressed air at one end, we observed air bubbles at the other end on some of the 0.37 m segments, but no air bubbles were observed on any 0.41 m segments. These results indicate that the vessel length of these rubber trees was less than 0.41 m and occasionally longer than 0.37 m. Only the segments 0.37 m long without vessels cut open at both ends were measured for xylem vulnerability to cavitation.

Xylem vulnerability to cavitation of branches was assessed using two techniques: Cavitron and air-injection with 0.37 m branch segments (Table 1). Both techniques gave sigmoid vulnerability curves with similar values of P_{12}, P_{50} P_{88} and s. However, a wider variation between repetitions was observed when using the air-injection technique.

The results for effects of sample properties on vulnerability to cavitation are shown in Table 1. The average P_{50} values of branches were −1.86, and −1.87 MPa for the 0.37 and 0.41 m segments, and −0.93 MPa for the 0.27 m long segments. Branches of the current year had a significant lower P_{50} than those of the previous year (−1.77 *vs.* −1.88 MPa, respectively), showing an effect of sample age. Branches fully exposed to sunlight and shaded branches had similar P_{50} values (−1.90 and −1.88 MPa, respectively).

Variability of xylem vulnerability to cavitation on branches of *H. brasiliensis*

The branch P_{50} values of the ten rubber clones studied were not statistically different, and ranged from −1.73 to −2.02 MPa (Figure 1*A*). No significant difference was found in P_{12} and P_{50} measured on branch samples between the three locations (Khon Kaen, Nong Khai and Surat Thani), and significant differences were observed only between the most sharply contrasting values of P_{88} at Nong Khai and Surat Thani (−2.63 ± 0.11 and −2.28 ± 0.09 MPa, respectively) (Figure 1*B*).

Table 1: Xylem vulnerability to cavitation (P_{50}) obtained from two analytical methods and different sample properties.

Conditions	P_{50} (MPa)	*n*	sample properties
Technique			
• Cavitron	−1.86 (0.05)	5	0.37 m long, current year branches from PB 235, RRIM 600 and RRIT 251, fully exposed to sunlight.
• Air-injection	−1.89 (0.15)	4	
Cavitron: Sample ages			
• Current year branch	−1.77 [a] (0.04)	5	0.37 m long branches from RRIM 600 and RRIT 251, fully exposed to sunlight.
• Previous year branch	−1.88 [b] (0.01)	6	
Cavitron: Sunlight exposure			
• Shaded	−1.88 (0.03)	5	0.37 m long, current year branches of PB 235.
• Sun-exposed	−1.90 (0.03)	5	
Cavitron: Sample size			Branches of current year and fully exposed to sunlight from:
• 0.27 m	−0.94 [a] (0.04)	4	-RRIM 600 with PB 217.
• 0.37 m	−1.86 [b] (0.02)	14	-Ten clones (BPM 24, PB 217, PB 235, PB 260, PB 5/51, RRII 105, RRII 118, RRIM600, RRIT 251 and RRIT 408).
• 0.41 m	−1.87 [b] (0.04)	24	-RRIM 600 and RRIT 251.

The variation in water potential inducing 50% loss of conductivity (P_{50}) was measured for branches from seven to ten years old rubber trees grown at the Surat Thani Rubber Research Centre in the southern region of Thailand (9°40'27"N, 99°6'40"E; altitude 19 m). Branches were collected from three to four individual trees for each test and each condition. The comparisons were carried out for the measurement techniques (Cavitron *vs.* air-injection), the sampling conditions: sample ages (current year and previous year branches) and sunlight exposure (shaded and sun-exposed), and the effects of sample size (0.27, 0.37 and 0.41 m). Data are mean values with standard errors in brackets. Variable *n* is the number of replications. Different letters indicate significant differences between means at 95% confidence interval.

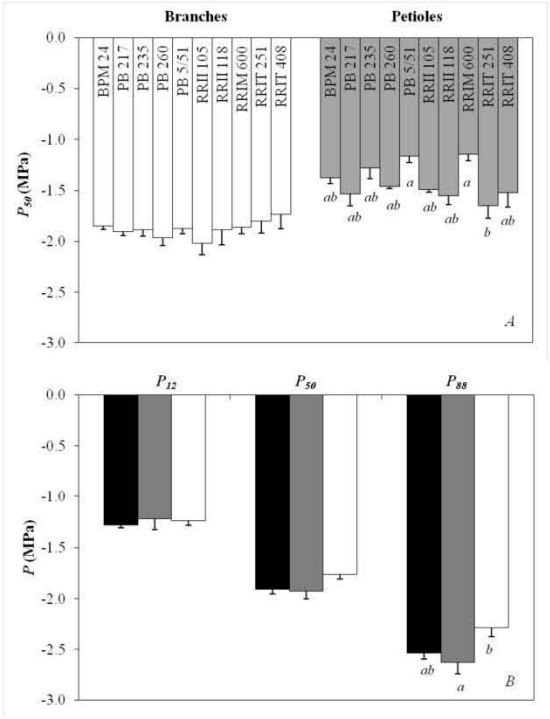

Figure 1: Clonal and environmental variabilities of xylem vulnerability to cavitation in rubber clones. P_{50} was measured on (*A*) branch and petiole samples from ten rubber clones from the same plantation at Nong Khai and (*B*) branches of RRIM 600 and RRIT 251 collected from three locations in Thailand with different annual rainfall (in brackets): Khon Kaen (1,000–1,200 mm; black), Nong Khai (1,400–1,600 mm; grey) and Surat Thani (2,800–3,200; white). The values are clonal average (n = 3 to 4) with standard error bars. Different letters indicate significant differences at 95% confidence interval.

Clone response to drought stress

The progression of drought stress was monitored by relating SWC and Ψ_{md} (Figure 2). SWC declined progressively after watering was withheld (Figure 2*A*), and significant differences were found between stressed and control trees from week 7 onward, whereas no significant difference was found between clones at any given time. Significant differences in Ψ_{md} were only observed on weeks 9 and 11 for the clones, with the most sharply contrasting Ψ_{md} values (Figure 2*B*).

The drought response of rubber trees was investigated for stomatal regulation ($g_s/g_{s\,max}$), embolism rate (PLC) and leaf shedding (LS) (Figure 3). Before the drought stress, the $g_s/g_{s\,max}$ ranged from 83.60 to 99.76%, and after the application of drought stress, declining trends in $g_s/g_{s\,max}$ were observed (Figure 3*A*). The differences in $g_s/g_{s\,max}$ between clones were not significant except between RRIT 251 (110.48% ± 7.15) and RRII 118 (36.21% ± 18.83) on week 2 after watering was withheld. During the drought period, the PLC of the petioles increased (Figure 3*B*) and significant differences were found only between PB 5/51, RRIT 251 and RRIT 408 (PLC = 60.25 to 61.50%) compared with BPM 24 (26.20%) on week 6. By week 11 of drought stress, high embolism rate occurred for all the clones (PLC = 74.98 to 87.07%). The relative shed leaf area (LS, %) shows the kinetics of leaf shedding of the clones (Figure 3*C*). On weeks 9 and 11, significant differences were found in LS between clones; BPM 24 clearly shed a larger leaf area than the other clones.

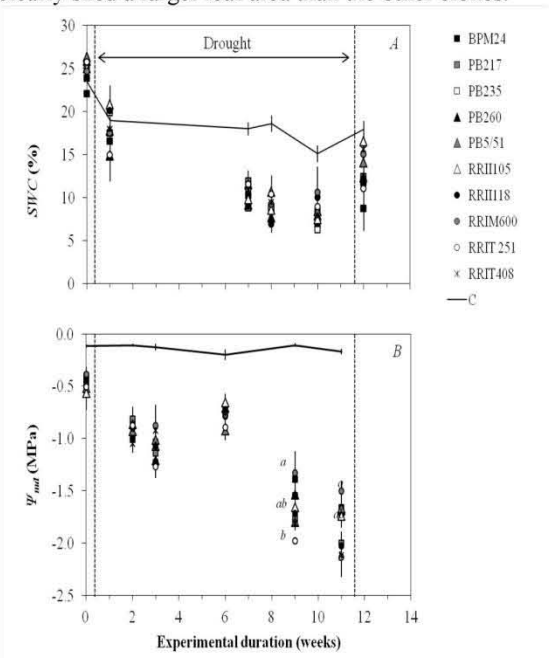

Figure 2: Survey of soil water content (SWC) and midday leaf water potential (Ψ_{md}) in 10 rubber clones during a drought. The solid lines indicate mean values measured on control trees (n = 30) and dots are mean values measured on stressed trees (n = 3). Bars are standard errors. Different letters indicate significant difference between clones at a given time point of the kinetics, with 95% confidence interval.

Significant differences were found in means of petiole P_{50} between the clones, with a greater variation (from −1.15 to −1.65 MPa) than that in branch P_{50} (Figure 1*A*). The petioles of PB 5/51 and RRIM 600 were the most vulnerable to cavitation, whereas petioles of RRIT 251 were the least vulnerable. Petioles were still more vulnerable to cavitation than branches, with significant differences (at 95% confidence interval) when comparing the branch P_{50} values with petiole ones, except for RRII 118, RRIT 251 and RRIT 408. In Figure 4, vulnerability curves of branches and petioles are presented for RRIM 600 and RRIT 251, which had the most sharply contrasting petiole P_{50} values.

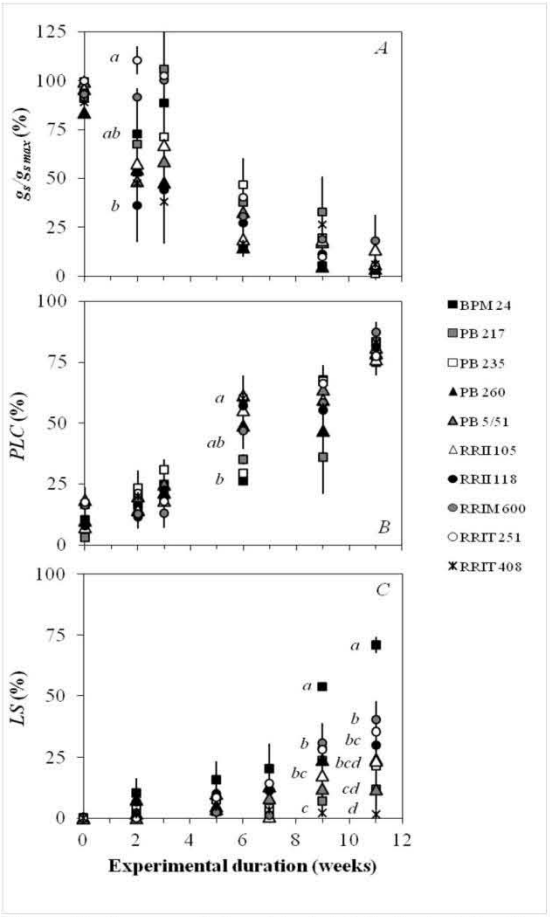

Figure 3: Survey of drought-related traits in 10 rubber clones during a simulated drought (A) Relative stomatal conductance (gs /gs max), (B) percentage loss of petiole xylem conductivity (PLC) and (C) relative shed leaf area (LS) were plotted versus drought duration (weeks). Dots represent the means (n = 3) and the bars are standard errors. Different letters indicate a significant difference between the clones at a given point of the kinetics, with 95% confidence interval.

The $g_{s\,50}$ (MPa) and LS_{50} are shown in Table 2 along with the calculated safety margins of petioles and branches. The ten clones had similar $g_{s\,50}$, but different LS_{50} values. The petiole safety margin was not significantly different between clones, whereas the safety margin of branches ranged from −1.02 to 0.54 MPa, with significant differences between PB 217 and RRIT 408 with other clones.

Before the watering was withheld, the ranges of tree height for control and stress group were 2.67–4.49 m and 3.45–4.65 m, respectively. Significant differences were found for tree height between clones in the stress group, RRIM 600 trees being significantly taller (4.65 m) than PB 217, PB 260, PB5/51 and RRIT 118 trees ($\bar{x}$ = 3.47 m ± 11.93). Before drought treatment, the GR in height ranged from 0.048 to 0.179 m.d^{-1}, with no significant difference between clones. During the dry period, the GR was reduced in both control and stress conditions. The GR of control trees ranged from 0.003 to 0.122 m.d^{-1}, while the stressed trees had GR ranging from 0.004 to 0.081 m.d^{-1}, and no significant difference was observed either between clones or between watering treatments.

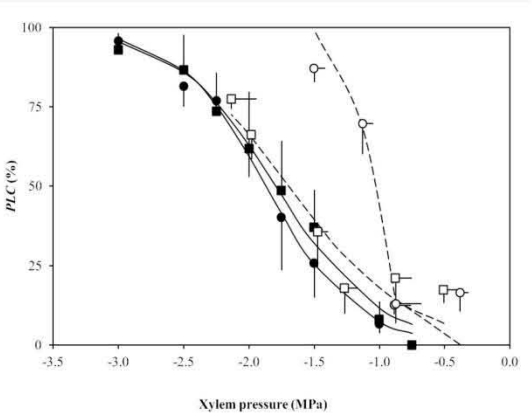

Figure 4: Xylem vulnerability curves of samples of branches (closed symbols) and petioles (open symbols) from RRIM 600 (circle) and RRIT 251 (square). Data are means values (n = 3 or 4) and bars are standard errors. Logistic lines were fitted to data using a sigmoid function.

Discussion

Xylem vulnerability to cavitation suggests itself as a suitable criterion for screening drought-tolerant clones, because it is considered as a critical trait for tolerance to severe drought and can be rapidly assessed on branches of rubber trees using the Cavitron centrifuge technique and the air-injection technique. However, the reliability of such techniques has to be tested prior to any investigations on a species. Sigmoid vulnerability curves were obtained from both techniques when using samples longer than the maximum vessel length (0.37 m or 0.41 m), whereas the 0.27 m segments contain vessels that were open at both ends, thus resulting in vessels abnormally vulnerable to cavitation. This results from the 'open vessel' artifact in vulnerability to cavitation (Choat *et al.*, 2010; Cochard *et al.*, 2010; Delzon and Cochard, 2014). The effects of age, sunlight conditions, sample size and location observed here and in other studies with different species (Cochard *et al.*, 1999; Cochard *et al.*, 2007; Herbette *et al.*, 2010) highlight the importance of sample design when investigating vulnerability to cavitation. To pursue the investigation of the clonal variability and environmental plasticity of xylem vulnerability to cavitation, we thus performed the measurement on branch samples from the current year, at least 0.37 m long and grown under full sunlight conditions.

Table 2: Hydraulic safety margins for petiole and branch of ten rubber clones. Values are means (n = 3) with standard error in the brackets. Different letters indicate significant differences between clones at 95% confidence interval.

Clone	$g_{s\,50}$ (MPa)	Petiole P_{50} (MPa)	Petiole safety margin (MPa)	LS_{50} (MPa)	Branch P_{50} (MPa)	Branch safety margin (MPa)
BPM 24	−0.61 (0.05)	−1.37 ^{ab} (0.06)	0.77 (0.02)	−1.30 ^{a} (0.08)	−1.85 (0.04)	0.54 ^{a} (0.08)
PB 217	−0.96 (0.05)	−1.53 ^{ab} (0.12)	0.57 (0.08)	−2.86 ^{b} (0.08)	−1.91 (0.04)	−0.95 ^{b} (0.08)
PB 235	−1.14 (0.08)	−1.28 ^{ab} (0.11)	0.14 (0.16)	−1.68 ^{a} (0.16)	−1.88 (0.07)	0.20 ^{a} (0.16)
PB 260	−0.91 (0.21)	−1.46 ^{ab} (0.03)	0.55 (0.18)	−1.85 ^{a} (0.11)	−1.96 (0.08)	0.11 ^{a} (0.11)
PB 5/51	−0.94 (0.06)	−1.16 ^{a} (0.06)	0.22 (0.02)	−2.06 ^{ab} (0.17)	−1.87 (0.05)	−0.18 ^{a} (0.17)
RRII 105	−0.92 (0.08)	−1.49 ^{ab} (0.03)	0.57 (0.08)	−1.87 ^{a} (0.13)	−2.02 (0.11)	0.15 ^{a} (0.13)
RRII 118	−0.84 (0.17)	−1.55 ^{ab} (0.09)	0.71 (0.20)	−1.68 ^{a} (0.12)	−1.89 (0.15)	0.20 ^{a} (0.12)
RRIM 600	−0.98 (0.13)	−1.15 ^{a} (0.06)	0.17 (0.07)	−1.51 ^{a} (0.10)	−1.86 (0.06)	0.35 ^{a} (0.10)
RRIT 251	−1.35 (0.25)	−1.65 ^{b} (0.13)	0.30 (0.33)	−1.62 ^{a} (0.27)	−1.80 (0.12)	0.18 ^{a} (0.27)
RRIT 408	−0.91 (0.10)	−1.52 ^{ab} (0.14)	0.61 (0.04)	−2.75 ^{b} (0.20)	−1.73 (0.14)	−1.02 ^{b} (0.20)

Branch and petiole xylem vulnerability to cavitation of rubber trees found in this study were in the same range as those previously found by Sansing *et al.* (2004), although the levels of vulnerability between the two organs were not in agreement. We found no difference between clones for the vulnerability to cavitation of branches, even though the clones were shown to be different for many other traits related to growth, crown architecture and latex yield (Besse *et al.*, 1994; Chandrashekar *et al.*, 1998; Lekawipat *et al.*, 2003; Priyadarshan *et al.*, 2005). The phenotypic plasticity for vulnerability to cavitation also appeared very weak for this species when comparing trees grown under contrasting environmental conditions (drought-prone versus traditional areas), as well as with different conditions of sunlight or sample age. Taken together, our results on this crop species support a genetic canalization for the vulnerability to cavitation. This canalization for xylem vulnerability to cavitation was clearly demonstrated in populations of a forest species (Lamy *et al.*, 2011). Nevertheless, there were differences in vulnerability to cavitation between clones when considering the petiole, which is not a critical organ like the branch that carries the buds needed for survival. In addition, vulnerability to cavitation for the petiole is higher than in branches, and the difference between them varies depending on the clones. Higher vulnerability of xylem vessels in petioles than in branches indicates vulnerability segmentation, also found in other species (Tsuda and Tyree, 1997; Tyree *et al.*, 1993). The xylem conduits in petioles being vulnerable to cavitation, they can become fully embolized at fairly high water potential, which leads to a hydraulic disconnection between leaf and branch, and weakens the tension in the branch. This ability prevents hydraulic failure in the branches and the stems, ensuring adequate water supply to buds (Barigah *et al.*, 2013). More vulnerable conduits of petiole in rubber trees may thus be considered as an advantage for tree survival rather than a drawback. We would expect the genetic canalization for the vulnerability to cavitation to hold for critical organs bearing buds, and that the xylem vulnerability to cavitation could vary in less critical organs, as for other traits we measured here or traits measured in previous studies. This hypothesis now needs to be thoroughly investigated by testing the respective variability for branch and petiole in several species.

In addition to xylem vulnerability to cavitation, there are other traits that can delay drought-induced cavitation events in xylem. To compare clones for response to drought, we followed stomatal conductance, petiole embolism and leaf shedding for the ten clones during a drought (Figure 4), and the safety margins of petioles and branches were then calculated (Table 2). We found clear differences between clones for the stomatal closure at weeks 2 and 4, then for the embolism level in petiole at week 6, and later for the level of leaf shedding starting from week 9. The stomatal regulation when facing drought stress indicates isohydric behavior in the rubber tree. This finding is consistent with previous reports (Isarangkool Na Ayutthaya *et al.*, 2011; Kobayashi *et al.*, 2014; Kunjet *et al.*, 2013). Although the stomatal regulation delays the occurrence of critical embolism, particularly during the onset of drought stress, it cannot completely prevent cavitation occurring, and thus it cannot be sufficient, especially in conditions of severe drought. Embolism in petioles and leaf shedding would also help in preventing hydraulic failure in branches and stem. We observed that leaf shedding after the embolism rate in petiole increased significantly, suggesting that high embolism rate would lead to leaf shedding. However, we cannot rule out the possibility that the two events are unrelated, even if both contribute to preventing hydraulic failure in branches. The petiole safety margin of 10 rubber clones ranged from 0.14 to 0.77 MPa, but there was no significant difference between them. The safety margin of branches varied from −1.02 to 0.54 MPa, and there were significant differences between mean values of PB 217 and RRIT 408 with the other clones. The

negative or near-zero safety margins indicate that rubber trees of some clones have kept their stomata opened and their leaves attached within the full range of endurable water potential before failure of the hydraulic system. Taken together, these results show that there are clonal differences for some traits related to avoidance of drought-induced cavitation.

It is difficult to compare clones for their drought tolerance in this study, and thus the respective contribution of the different traits. The effect of drought stress on tree growth is not very apparent, and the trees are too young to compare for their latex yield. The comparison of GR between the stressed trees and the control trees showed a non-significant difference. The reason for this similar GR between control and stressed trees may be because the experiment was done during a dry season in Thailand, which is a period when the growth of the rubber tree is normally halted (Chandrashekar *et al.*, 1998). To address this problem, further research is needed with a more severe drought and more individual trees to investigate the recovery ability.

To conclude, our study reveals that xylem vulnerability to cavitation in branches is not a strong criterion in selecting for drought tolerance in the rubber tree population studied. The variation for this trait is weak. Trade-offs between vulnerability to cavitation and growth-related traits were shown in other species (Cochard *et al.*, 2007; Wikberg and Ögren, 2004). Here, we found for rubber trees that vulnerability to cavitation of branches did not vary in clones, whereas these largely differed in their growth and latex yield. This means that the selection programs for agronomic traits did not affect xylem vulnerability to cavitation. Further research on xylem vulnerability to cavitation should be carried out with larger populations including Brazilian rubber trees to broaden the genetic diversity beyond the widespread commercial clones. Nevertheless, considering the differences in stomatal regulation, petiole vulnerability to cavitation and leaf shedding, which indicate different desiccation avoidance behaviors between the clones, screening for drought resistance should firstly focus on these traits. The combination of the scion and the rootstock would also be a next step in investigating the drought responses of this species, especially because knowledge of rootstock responses to drought stress is still lacking for rubber trees.

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III. Genetic variation in plant response to drought stress among five apple genotypes from a progeny of 'Starkrimson' x 'Granny Smith'.

Results

1. Vulnerability to cavitation of the non-stressed apple trees
2. Responses of the five apple genotypes to drought stress

Discussion

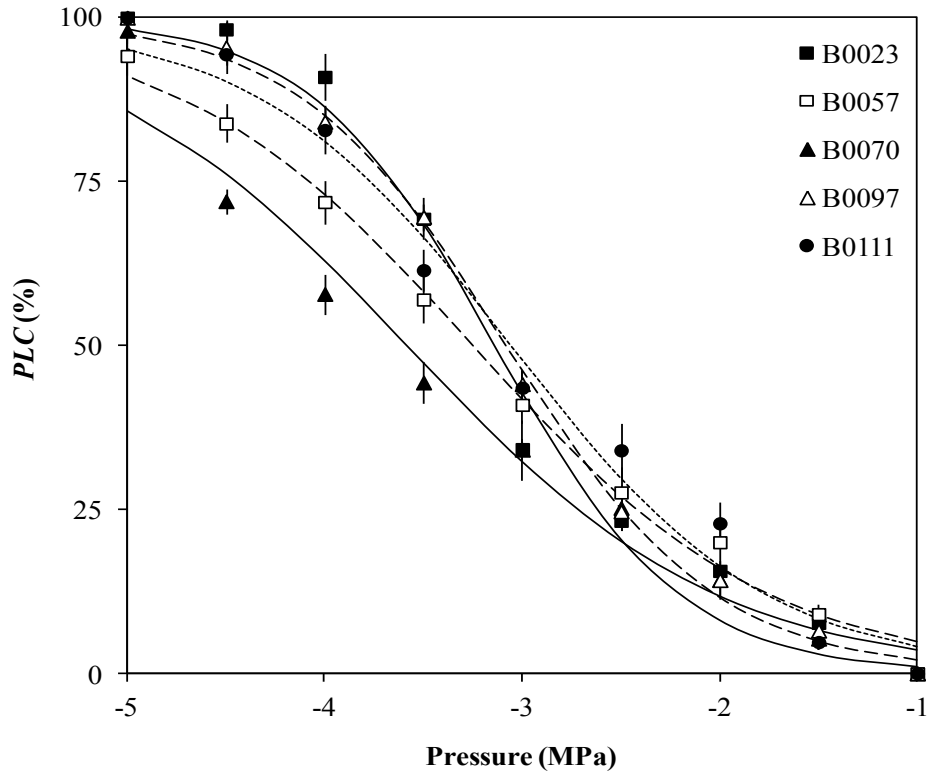

Figure 37. The vulnerability curves (*VC*) measured on stems of five apple genotypes.

The measurement was carried out with the Cavitron technique on 0.37 m long stem segments of five apple genotypes: B0023, B0057, B0070, B0097 and B0111. The loss of xylem conductivity (*PLC*, %) was plotted versus xylem pressure (MPa) and fitted with the sigmoidal function (Pammenter and Van der Willigen, 1998) to obtain the *VC*. Dots are means of 4 samples and error bars are *SE*.

Table 7. Vulnerability to cavitation traits for five genotypes of apple tree.

Genotype	P_{12} (MPa)	P_{50} (MPa)	P_{88} (MPa)	S (%/MPa)
B0023	−2.21 (±0.17)	−3.14 (±0.09) [a]	−4.08 (±0.19) [a]	54.47 (±8.56) [a]
B0057	−1.75 (±0.47)	−3.25 (±0.19) [ab]	−4.76 (±0.36) [b]	34.78 (±8.59) [c]
B0070	−2.03 (±0.42)	−3.58 (±0.18) [b]	−5.13 (±0.11) [c]	32.87 (±5.54) [c]
B0097	−2.02 (±0.23)	−3.08 (±0.13) [a]	−4.15 (±0.13) [a]	47.46 (±5.96) [ab]
B0111	−1.78 (±0.29)	−3.05 (±0.16) [a]	−4.32 (±0.16) [a]	39.99 (±6.19) [bc]

The xylem pressure causing 12%, 50% and 88% loss of xylem conductivity (P_{12}, P_{50} and P_{88}) and the slope of the *VC* (*s*) are shown. Values are means (n = 4) with *SE* in the brackets and different letters indicating significant differences between genotypes at 95% confidence interval.

The study of plant response to drought stress was carried out on five apple genotypes: B0023, B0057, B0070, B0097 and B0111, a cross of 'Starkrimson' x 'Granny Smith'. In a previous study of Lauri *et al.*, (2011), these five genotypes displayed contrast behaviors in drought tolerance mechanisms including the cavitation resistance and the water saving behavior. In this study, the vulnerability to cavitation was firstly measured on non-stress stem samples to verify whether the genetic variability of this trait which was found from the previous studyof Lauri *et al.* (2011) has been still preserved. Then, plant responses to drought stress were investigated. This experiment was conducted in two growing seasons of 2012 and 2013 where trees were grown with the irrigation at full field capacity (0.50 liter of water/tree, twice a week) and with half of the irrigation previously given (0.25 liter of water/tree, twice a week), respectively. The reduction of irrigation in the latter year was aimed to increase the possibility to observe the differences in plant responses to drought stress between genotypes. Trees were grown with these irrigation schemes for three and four month respectively before the irrigation was withheld in the stressed trees. The soil was left to dry down for 31 days for the experiment in 2012 and 33 days in 2013. The changes in soil water content (*SWC*), stomatal conductance (g_s), stem embolism rate (*PLC*) and growth of tree in height (*RGR*) and stem diameter were measured along the time progression.

Results

1. Vulnerability to cavitation of the non-stressed apple trees

Xylem vulnerability to cavitation was measured on non-stressed stems of apple trees. After stem was harvested; it was divided into two parts. The distal part immediately used for native embolism measurement while the proximal part successively used for vulnerability to cavitation measurement using Cavitron.

The average native embolism of five genotypes ranked from 5.03% to 9.23% with mean value of 6.96% *SE* ±1.42. There was no significant difference found between means of the five genotypes. These results ensured that the following measurement of vulnerability to cavitation on the proximal part will be conduct on conductive stems and if the differences in cavitation resistance were found; it would due to the genetic variation between the five genotypes.

Table 8. Leaf and stem traits for five apple genotypes for the experiment in 2012 and 2013.

Year	Genotypes	Number of leaves (number tree^{-1})	Leaf size (cm^2)	Total leaf area (m^2)	*LMA* (g m^{-2})	Stem height (m^2)	Stem diameter (mm)
2012	B0023	40.33 (±2.96)	43.40 (±3.28) ^{ab}	0.177 (±0.027)	58.50 (±1.03)	1.58 (±0.10) *	12.07 (±0.38)
	B0057	38.33 (±0.88)	35.29 (±0.54) ^{c}	0.135 (±0.005) *	60.80 (±1.52)	1.50 (±0.02) *	11.40 (±0.70)
	B0070	39.00 (±1.08) **	36.36 (±1.81) ^{c}	0.141 (±0.004)	59.11 (±1.03)	1.51 (±0.02) *	11.30 (±0.46)
	B0097	34.00 (±1.53)	49.20 (±1.55) ^{a}	0.167 (±0.011) *	61.95 (±0.78)	1.59 (±0.05) *	10.20 (±0.31)
	B0111	35.00 (±2.65)	38.10 (±1.52) ^{bc}	0.133 (±0.010)	62.83 (±0.77)	1.56 (±0.08) *	10.97 (±0.57)
2013	B0023	38.33 (±0.88) ^{a}	35.29 (±0.54)	0.134 (±0.008)	55.12 (±1.97)	0.97 (±0.04) **	10.02 (±0.23) ^{ab}
	B0057	37.67 (±2.19) ^{a}	44.06 (±6.24)	0.111 (±0.005) **	60.72 (±6.00)	0.97 (±0.01) **	10.78 (±0.38) ^{a}
	B0070	28.67 (±0.88) ^{b} *	46.74 (±2.12)	0.127 (±0.009)	65.55 (±0.83)	0.85 (±0.01) **	11.73 (±0.42) ^{a}
	B0097	31.67 (±1.20) ^{b}	40.46 (±3.96)	0.133 (±0.004) **	59.60 (±1.58)	0.87 (±0.05) **	8.80 (±0.66) ^{b}
	B0111	30.00 (±2.08) ^{b}	40.77 (±3.29)	0.123 (±0.017)	57.74 (±1.45)	0.89 (±0.04) **	10.00 (±0.21) ^{ab}

The number of leaves, leaf size, total leaf area and stem height of five apple genotypes: B0023, B0057, B0070, B0097 and B0111 are presented. Values are means (n = 3 − 4) with *SE* in the brackets. Significant differences between genotypes at 95% confidence interval are indicated with different letters whereas the asterisks show significant differences found between two experimental years.

The proximal part of stem was re-cut to the length of 0.37 m segment for the Cavitron. This length was sufficient to obtain intact vessels at the center. It was confirmed by a negative observation of air bubbles when the segment has been infiltrated by compressed air. The *VC*s obtained from stem segments were sigmoid for all genotypes (Figure 37). The features of vulnerability to cavitation of five genotypes: P_{12}, P_{50} and P_{88} and slope of the *VC* (*s)* were calculated (Table 7). The P_{12} of five genotypes was ranked from −1.75 to −2.21 MPa. There was no significant difference found between the means at 95% confidence interval while there were significant differences found between means of P_{50}, P_{88} and *s*.

2. Responses of the five apple genotypes to drought stress

For the experiment in 2012, there were similarity of leaf and stem straits in five genotypes of trees while in the experiment in 2013, there were significant differences found on number of leaves and stem diameter between genotypes at 95% confidence interval (Table 8). Comparing the two experimental years, there were significant differences found between means of five genotypes on some traits ($P < 0.05$), including the number of leaves of B0070, the total leaf area of B0057 and B0097 and the height of stem for all genotypes. The interested hydric traits have been measured before the drought stress was applied. These values were used as references and were labeled as day 0 in the following figures and tables.

In the experiment of 2012 and 2013 respectively, *SWC* of the studied apple genotypes was ranked from 70.30% to 76.03% and 42.81% to 52.65% before the irrigation was withheld. There were no significant differences of genotypes in both experiments. At the later periods of time, soil was dried down by the tree transpiration. At the same period of time of the experiment, there were no significant differences ($P > 0.05$) in *SWC* between the genotypes except on day 24th and 31st of the drought stress in 2012 experiment. Therefore, the *SWC* (Figure 38) was presented as mean values of the five genotypes. From day 17th onward, *SWC* became rather constant in both experiments, with value ranked from 24.39% to 27.47% and from 26.24% to 30.23% for the 2012 and 2013experiment respectively. On the last day of the drought stress, *SWC* reached the values of 24.39 and 26.24% for the former and latter year.

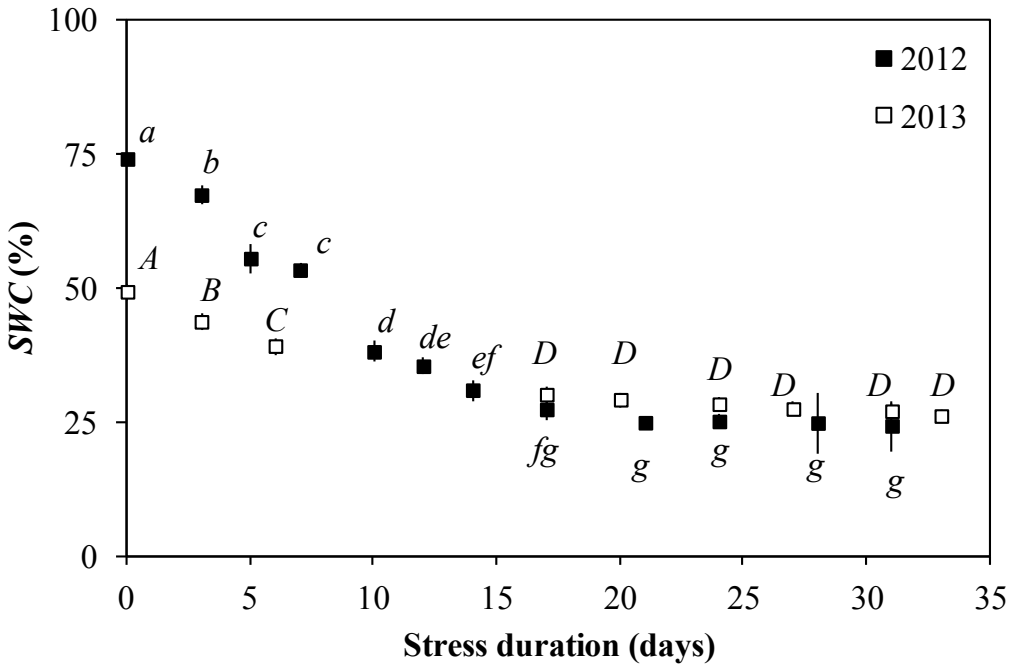

Figure 38. Changes in soil water content (*SWC*, %) during the drought experiments in 2012 and 2013.

During the time of growing, 0.50 and 0.25 liters of water were applied to each tree twice a week for the experiment in 2012 and 2013 respectively. After the day '0' reference measurement, irrigation was completely withheld from stressed trees and soil was left to dry down for 31 days in 2012 and 33 days in 2013. The decrement in *SWC* was observed as the time progress with significant differences ($P < 0.05$) between days. The averaged values (n = 15 − 20) from studied five genotypes (B0023, B0057, B0070, B0097 and B0111) were presented with standard error bars. Different letters present significant differences between the measuring days. The minuscule and the majuscules letters show the differences between days for the experiment in 2012 and 2013 respectively.

While apple trees were receiving the irrigation regularly, midday leaf water potential (Ψ_{md}) of five genotypes was ranked from −0.34 to −0.43 MPa and −0.89 to −1.06 MPa for experiment in 2012 and 2013 respectively. There was no significant difference found between genotypes in both years ($P > 0.05$). However, Ψ_{md} of five genotypes was significantly lower in 2013 as a result of less irrigation when compared to the experiment in 2012. Although the duration of drought stress was lasted for 31 days for the experiment in 2012, Ψ_{md} was only measured for 24 days. This described that the 24-day measurement Ψ_{md} had nearly reached the value of −10 MPa, the maximum pressures applicable to the pressure chamber used in this study.

When the irrigation stopped, reduction in Ψ_{md} was observed along the progression of time (Figure 39). For the experiment in 2012, there were significant differences ($P < 0.05$) found between genotypes on day 12th, 14th, 17th and 24th of the drought stress. The genotype B0070 significantly reached lower Ψ_{md} when compared to the other four genotypes on day 12th and 14th of the drought stress (Ψ_{md} = −3.39 ±0.05 and −3.47 ±0.11 MPa, respectively). On day 17th, the genotype B0023 obtained significantly higher Ψ_{md} (−2.75 ±0.14 MPa) while on day 24th, the Ψ_{md} of B0111 (−9.71 MPa) was significantly lower when compared to other genotypes. In 2013 experiment, there were significant differences in Ψ_{md} between genotypes found on day 21st and 33rd of the drought stress while the Ψ_{md} of B0023 obtained significantly higher when compared to other genotypes.

The stomatal conductance (g_s) of five genotypes before drought stress was ranked from 249.54 to 396.67 mmol m^{-2} s^{-1} and from 276.40 to 326.40 mmol m^{-2} s^{-1} for the experiment in 2012 and 2013 respectively. The significant difference was not found either between the genotypes or between the two experimental years. When compared changes in stomatal regulation between the genotypes, the relative stomatal conductance ($g_s/g_{s\,max}$) was calculated. As the drought progressed, a declining trend in $g_s/g_{s\,max}$ was observed for all genotypes. While significant differences at 95% confidence interval were mostly observed on the $g_s/g_{s\,max}$ between the genotypes for the experiment in 2012, significant difference was not found in the experiment of 2013 on a given day of the drought stress (Table 9). The $g_s/g_{s\,max}$ was plotted against Ψ_{md} and data was fitted with sigmoid function (Pammenter and Van der Willigen, 1998) as indicated in equation *14*. The calculation of xylem tensions (MPa) which caused 12, 50 and 88% of stomatal closure ($g_{s\,12}$, $g_{s\,50}$ and $g_{s\,88}$, respectively) (Table 10). Significant differences were found only on $g_{s\,50}$ between the five apple genotypes in 2012, in

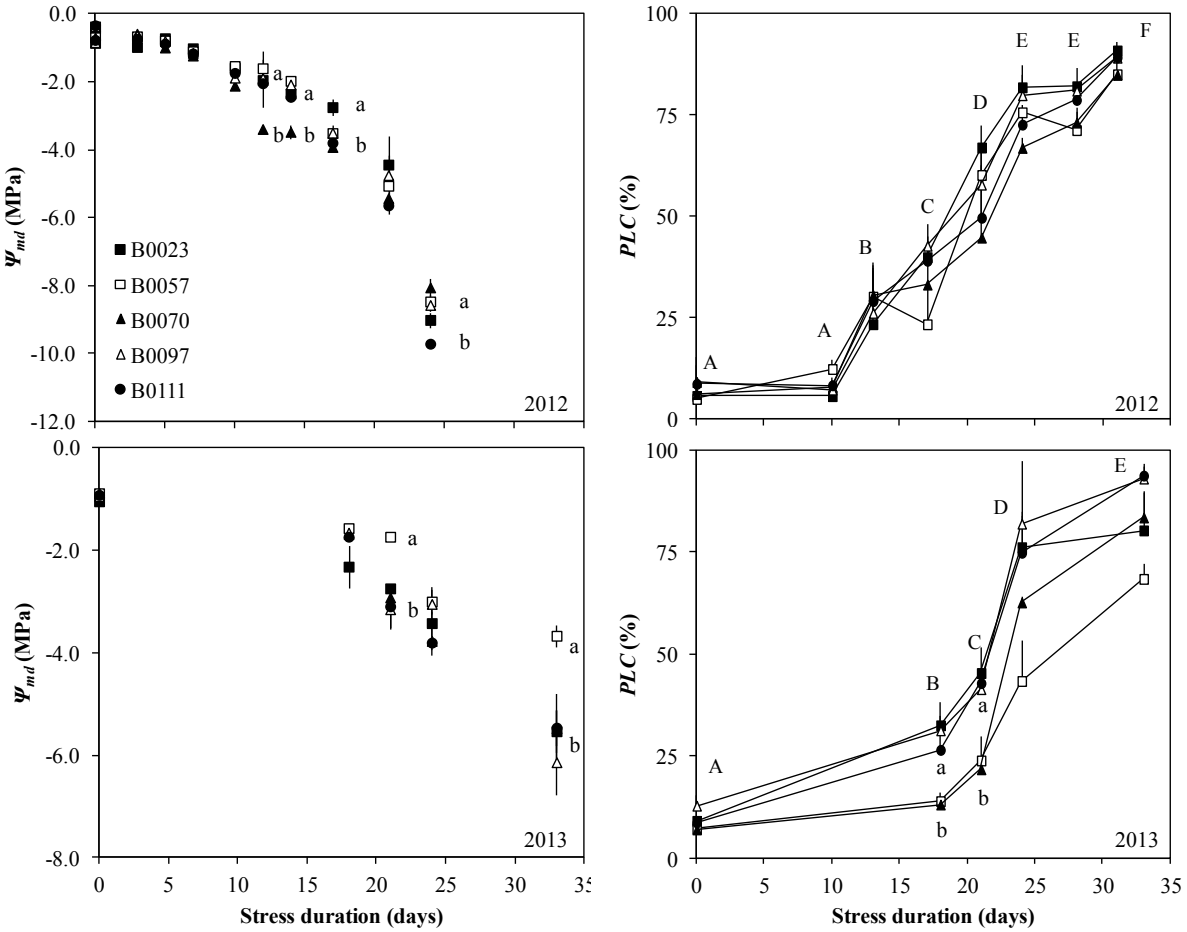

Figure 39. Evolutions of midday leaf water potential (Ψ_{md}, MPa) and stem embolism (*PLC*, %) of five apple genotypes during the drought experiment in 2012 and 2013.

Surveys of Ψ_{md} and stem *PLC* as a function of drought stress time (days) of the five apple genotypes studied (B0023, B0057, B0070, B0097 and B0111). Dots were mean values (n = 3) with *SE* as error bars. The values at day 0 had been measured before drought stress was applied. When the measurement on this day was finished, irrigation was withheld and the soil was left to dry down for 31 and 33 days for the experiment in 2012 and 2013 respectively. Different letters indicated significant differences at 95% confidence interval: the minuscule letters indicated the differences between the five genotypes whereas the majuscules showed the differences between the stress days.

which B0057 and B0097 had significantly higher $g_{s\ 50}$ when compared to the other three genotypes.

Before drought was applied, the stem embolism was low and there were significant differences ($P > 0.05$) between five genotypes. The averaged stem embolism of the five genotypes was 6.66 ± 1.39% and 8.92 ± 0.98% for the experiment in 2012 and 2013 respectively. Stem embolism increased as the drought progressed (Figure 39). In 2012 experiment, low stem embolism rate was maintained at the least until at day 10th of the drought where the averaged embolism of the five genotypes was 8.38 ± 0.89%. From day 13th onward, the stem embolism increased successively for all genotypes and on day 30th, the averaged stem embolism rate of the five genotypes reached approximately 87.9 ± 1.08%. Significant difference was not observed on the stem embolism rate between the five genotypes studied on the given experimental day. In 2013 experiment, the averaged stem embolism rose to 23.41 ± 3.52% on day 18th of the drought stress and there were significant differences ($P < 0.05$) found between the five genotypes. On this day, the genotypes B0057 and B0070 obtained significantly lower stem embolism when compared to the other genotypes. These differences between the genotypes were also found on day 21st of the drought stress. However, from day 24th onward, there was no significant difference found on the stem embolism between the genotypes. On day 33rd before the termination of drought experiment, the averaged stem embolism of the five apple genotypes was 83.66 ± 4.30%.

The hydraulic safety margin was calculated (Table 11) as the difference in pressure caused 88% of stomatal closure ($g_{s\ 88}$) and xylem pressure caused 12% or 50% loss of xylem conductivity in stem (P_{12} or P_{50}). They were named $g_{s\ 88} - P_{12}$ and $g_{s\ 88} - P_{50}$ respectively. The $g_{s\ 88}$ was obtained from the drought experiment in 2012 and 2013 while the P_{12} and the P_{50} were estimated with Cavitron technique. The $g_{s\ 88} - P_{12}$ and the $g_{s\ 88} - P_{50}$ from the 2012 experiment were negative for all the genotypes while positive values were found on the $g_{s\ 88} - P_{12}$ of B0023 and the $g_{s\ 88} - P_{50}$ of all the genotypes in the 2013 experiment. The differences between genotypes of both years for the respective traits were not significant at 95% confidence interval.

The effect of the drought stress on the tree growth was observed via the *RGR* (m d^{-1}), the change in relative growth over time. Here, we compared the *RGR* between the five genotypes studied and between control (C) and stressed (S) groups (Table 12). There was no significant difference found on the *RGR* between five apple genotypes within each group ($P > 0.05$) for

Table 9. Evolution in relative stomatal conductance ($g_s / g_{s\,max}$) of five apple genotypes during the drought experiment in 2012 and 2013.

Stress duration (days)		3	5	7	10	12	14	17	21	24
2012	**B0023**	76.99 (±3.74) ^{b}	82.9 (±7.69)	68.17 (±3.54) ^{a}	88.39 (±11.33) ^{a}	59.96 (±5.64) ^{a}	52.43 (±4.29) ^{ab}	55.00 (±5.44) ^{a}	33.15 (±4.48) ^{ab}	19.83 (±3.74) ^{bc}
	B0057	110.90 (±10.24) ^{a}	95.72 (±5.50)	54.40 (±3.00) ^{b}	67.08 (±3.89) ^{b}	45.33 (±7.93) ^{ab}	59.71 (±7.34) ^{a}	32.45 (±4.50) ^{b}	21.18 (±2.39) ^{bc}	12.67 (±1.98) ^{c}
	B0070	77.29 (±11.21) ^{b}	103.21 (±4.28)	78.04 (±5.08) ^{a}	64.10 (±8.02) ^{b}	30.81 (±3.37) ^{bc}	34.22 (±3.06) ^{c}	33.43 (±4.36) ^{b}	32.61 (±4.77) ^{ab}	37.26 (±2.84) ^{a}
	B0097	93.61 (±10.00) ^{ab}	86.82 (±2.94)	55.14 (±2.34) ^{b}	25.94 (±1.73) ^{c}	25.46 (±2.54) ^{c}	48.68 (±3.61) ^{abc}	21.31 (±3.14) ^{b}	38.59 (±10.16) ^{a}	23.37 (±3.39) ^{b}
	B0111	81.36 (±3.91) ^{b}	90.52 (±3.06)	74.19 (±3.39) ^{a}	71.41 (±3.27) ^{ab}	44.85 (±8.73) ^{ab}	43.93 (±5.10) ^{bc}	30.07 (±3.81) ^{b}	15.35 (±1.69) ^{c}	16.00 (±4.14) ^{bc}
Mean		**88.03 (±3.87) ^{A}**	**91.84 (±2.83) ^{A}**	**65.99 (±2.94) ^{B}**	**63.39 (±5.61) ^{B}**	**41.28 (±3.47) ^{CD}**	**47.79 (±2.65) ^{C}**	**34.45 (±3.24) ^{DE}**	**28.18 (±2.88) ^{EF}**	**21.83 (±2.39) ^{F}**
Stress duration (days)		**3**	**6**	**17**	**20**	**24**	**26**	**31**		
2013	**B0023**	80.73 (±4.73)	32.06 (±6.40)	28.86 (±3.51)	20.64 (±1.01)	10.66 (±1.99)	16.25 (±2.17)	2.57 (±0.20)		
	B0057	109.46 (±6.07)	52.80 (±5.66)	52.69 (±10.26)	23.68 (±2.61)	12.54 (±1.22)	24.45 (±2.63)	3.12 (±0.59)		
	B0070	111.84 (±8.41)	47.93 (±8.60)	29.98 (±5.78)	26.62 (±1.44)	12.81 (±1.85)	20.41 (±4.41)	3.68 (±0.67)		
	B0097	86.10 (±7.37)	54.78 (±4.98)	17.98 (±4.63)	26.15 (±1.63)	12.52 (±1.64)	7.89 (±1.97)	4.18 (±0.33)		
	B0111	97.79 (±5.67)	44.62 (±4.88)	34.26 (±5.64)	25.01 (±2.31)	6.36 (±1.93)	13.84 (±2.35)	4.53 (±0.84)		
Mean		**97.58 (±4.19) ^{A}**	**46.44 (±4.43) ^{B}**	**32.76 (±5.23) ^{C}**	**24.42 (±1.03) ^{D}**	**10.98 (±1.06) ^{E}**	**16.57 (±2.27) ^{E}**	**3.62 (±0.31) ^{F}**		

The $g_s / g_{s\,max}$ of B0023, B0057, B0070, B0097 and B0111 during the two drought experiments. It was computed using the g_s of stressed trees whereas the $g_{s\,max}$ was measured from control trees. Values were means (n = 9) with *SE* in the brackets. Different letters indicate significant difference at 95% confidence interval; the minuscule letters indicate the difference between five genotypes whereas the majuscules show the difference between the stress days.

the experiment in both years. However, there were significant differences found for this trait when compared *RGR* of C and S trees in 2012 on B0057, B0097 and B0111 as well as on B0057 in 2013.

Discussion

In this study, the five apple genotypes appeared to be more vulnerable to drought-induced cavitation when compared with the previous report of Lauri *et al.* (2011), except for B0023 which was less vulnerable (Table 13). In addition, the variation in xylem vulnerability to cavitation (P_{50}) between the five studied genotypes was much smaller when compared with the study of Lauri *et al.* (2011). The coefficient of variance (CV), a ratio of the standard deviation to the mean of the apple tree P_{50} was 0.39 in the previous study while, in this study, it reduced to 0.07. The narrow variation in P_{50} was rather disappointed since we chose these genotypes for the extent of genetic variability of this trait. Nevertheless, it was still possible to categorize the genotypes into two distinctive groups: the tolerant (B0057 and B0070) and the sensitive (B0023, B0097 and B0111).

It has already been reported that the differences in environmental conditions can induce variation in vulnerability to cavitation of the species (Herbette *et al*., 2010). This may explain the differences found for vulnerability to cavitation between the two studies since there was a large difference between the plant materials used. In the previous study, samples of the analysis of xylem vulnerability to cavitation were the annual 7-year-old branches, own-rooted trees planted in the field at the Melgueil INRA Montpellier Experimental Station (south-east of France). In this study, 4-month-old stems were used for the measurement. They were grafted trees planted in pots and cultivated under greenhouse conditions at Clermont-Ferrand. Indeed, a phenotypic plasticity for this trait was also observed in other studies (Awad *et al*., 2010; Corcuera *et al*., 2011). Moreover, it was also possible that different cavitation resistance might be the effect of rootstock. It is still uncertain whether the rootstocks can modify the xylem vulnerability to cavitation of the species. Some studies demonstrated that rootstock can modify hydric properties of the scion and resulted in different response to drought stress (Atkinson *et al*., 2003; Bauerle *et al*., 2011; Feng *et al*., 2011; Jones *et al*., 1989; Tramontini *et al*., 2013). However, this was not always the case for some other studies (Trifilò *et al*., 2007). While in the previous study of Lauri *et al.* (2011), sample branches were from own-rooted trees, M9 was used as a rootstock in this study. The M9 (Malling IX) rootstock was characterized as an early fertility and dwarfing rootstock. Although known to

Table 10. Features of stomatal regulation of five apple genotypes for the drought experiment in 2012 and 2013.

Year	Genotypes	$g_{s\ 12}$ (MPa)	$g_{s\ 50}$ (MPa)	$g_{s\ 88}$ (MPa)	*s* (% MPa)
2012	B0023	−0.60 (±0.25)	−3.73 (±0.06) [b]	−6.85 (±0.28)	16.22 (±1.30)
	B0057	0.55 (±0.08)	−2.23 (±0.30) [a]	−5.01 (±0.66)	18.54 (±2.13)
	B0070	−0.36 (±0.13)	−3.09 (±0.26) [b]	−5.81 (±0.54)	18.77 (±1.98)
	B0097	0.89 (±1.20)	−2.01 (±0.29) [a]	−4.90 (±1.75)	28.99 (±12.80)
	B0111	−1.10 (±0.15)	−3.05 (±0.20) [b]	−5.00 (±0.54)	27.56 (±5.55)
2013	B0023	−1.48 (±0.02)	−1.78 (±0.07)	−2.07 (±0.11)	177.69 (±24.08)
	B0057	−1.17 (±0.03)	−1.59 (±0.31)	−2.00 (±0.62)	426.80 (±246.48)
	B0070	−1.39 (±0.17)	−2.08 (±0.24)	−2.76 (±0.34)	77.90 (±15.35)
	B0097	−1.49 (±0.14)	−2.24 (±0.25)	−2.99 (±0.36)	69.81 (±10.19)
	B0111	−1.54 (±0.11)	−1.89 (±0.33)	−2.25 (±0.56)	434.87 (±298.91)

Xylem tensions (MPa) causing 12, 50 and 88% of stomatal closure ($g_{s\ 12}$, $g_{s\ 50}$ and $g_{s\ 88}$, respectively) of apple genotypes: B0023, B0057, B0070, B0097 and B0111 were obtained from the sigmoid function fitted to the relationship between relative stomatal conductance ($g_s/g_{s\ max}$) with the midday leaf water potential (Ψ_{md}). Values were means (n = 3) with *SE* in brackets. Different letters indicated significantly differences between genotypes at 95% confidence interval. Significant difference was not found between genotypes for the traits measured in 2013.

be sensitivity to water logging, relatively low frost hardiness and susceptible to drought, it was widely used in Europe because of the low shoot vigorous which made it suitable for high density planting.

In the study of Jones *et al.* (1989), higher vulnerable to cavitation was observed in a scion grafted on the dwarfing M9 rootstock compared with the scion grafted on vigorous rootstock M25. Similar trend of vulnerability to cavitation was also found in the study of Bauerle *et al*. (2011). The study indicated that scion grafted on the vigor rootstock was more tolerant to water deficit compared with the grafting on the dwarf one with higher resistance to cavitation. These results presumed an effect of a modification in xylem vessel diameter. In the former case, a reduction of scion vessel diameter was observed when tree was submitted to drought stress, while in the latter case of dwarfing rootstock, it did not substantially shift. The reduction in vessel diameter helped to increase conduit ressitance to cavitation (Awad *et al*., 2010; Hacke *et al*., 2001a; Jacobsen *et al*., 2005). Therefore, small variation in xylem vulnerability to cavitation and higher vulnerability found in this study compared to the study of Lauri *et al*. (2011) might be a result of the M9 rootstock concealing original adjustment ability of the scion genotypes to water deficit. From these results, further investigation is needed to verify the source of variability of xylem vulnerability to cavitation, particularly on the vulnerability to cavitation. Beyond the interest for this study, it is of fundamental and applied interest.

During the experiment of drought stress, tree transpiration caused depletion of the soil water which could be seen from the reduction of *SWC*, thus lower water potential in trees and exposed their xylem to the risk of cavitation and embolism. Despite similar reduction in *SWC*, the decrement of Ψ_{md} was differed between the genotypes. It suggested that the genotypes were different in transpiration rate as soil evaporation assumed to be nil from covered soil surface.

Isohydric behavior of the apple tree (Regnard *et al*., 2009) was confirmed by the observation of stomatal regulation; when facing drought stress, the apple trees closed their stomata to prevent excessive water loss through transpiration. However, as not all stomata were closed, stomatal regulation only helped to delay the occurrence of critical embolism in stem and stem embolism rate was continuously increased as time progress. Similar total leaf area and *LMA*

Table 11. Hydraulic safety margin of five apple genotypes.

Year	Safety margin (MPa)	B0023	B0057	B0070	B0097	B0111
2012	$g_{s\ 88} - P_{12}$	−4.65 (±0.25)	−3.27 (±0.66)	−3.87 (±0.54)	−2.88 (±1.75)	−3.24 (±0.44)
	$g_{s\ 88} - P_{50}$	−3.17 (±0.28)	−1.76 (±0.51)	−2.23 (±0.32)	−1.82 (±0.65)	−1.95 (±0.54)
2013	$g_{s\ 88} - P_{12}$	0.13 (±0.23)	−0.26 (±0.62)	−0.76 (±0.34)	−0.97 (±0.16)	−0.49 (±0.56)
	$g_{s\ 88} - P_{50}$	1.07 (±0.11)	1.25 (±0.44)	0.82 (±0.52)	0.09 (±0.36)	0.80 (±0.26)

The hydraulic safety margin (MPa) for the five apple genotypes (B0023, B0057, B0070, B0097 and B0111) were calculated as the differences in tension causing 88% of stomatal closure ($g_{s\ 88}$) and xylem pressure causing 12% or 50% loss of xylem conductivity (P_{12} or P_{50}). The $g_{s\ 88}$ was obtained from drought experiment while the P_{12} and the P_{50} were values estimated with the Cavitron. They were named $g_{s\ 88} - P_{12}$ and $g_{s\ 88} - P_{50}$, respectively. Values were means (n = 3) with *SE* in brackets.

Table 12. The relative growth rate (*RGR*) of control (C) and stressed (S) trees of five apple genotypes from the experiment in 2012 and 2013.

Year	Genotypes	*RGR* (m d^{-1})	
		C	S
2012	B0023	0.0075 (±0.0004)	0.0036 (±0.0016)
	B0057	0.0062 (±0.0007) [a]	0.0031 (±0.0002) [b]
	B0070	0.0067 (±0.0019)	0.0031 (±0.0002)
	B0097	0.0085 (±0.0009) [a]	0.0024 (±0.0007) [b]
	B0111	0.0068 (±0.0004) [a]	0.0017 (±0.0009) [b]
2013	B0023	0.0065 (±0.0014)	0.0020 (±0.0013)
	B0057	0.0100 (±0.0022) [a]	0.0032 (±0.0010) [b]
	B0070	0.0058 (±0.0008)	0.0066 (±0.0008)
	B0097	0.0061 (±0.0002)	0.0054 (±0.0017)
	B0111	0.0069 (±0.0013)	0.0048 (±0.0010)

The relative growth rate (*RGR*) of B0023, B0057, B0070, B0097 and B0111; it was calculated from the relative growth in stem height over the duration of drought stress. The values were averaged (n = 3) with *SE* in the brackets. Different letters indicated significant difference at 95% confidence interval when compared *RGR* to control (C) and stressed (S) trees from the same genotype.

of the five genotypes studied suggested that the differences in stomatal regulation were results of genetic variation in stomatal sensitivity to drought stress of the genotypes.

In 2012 experiment, B0023 was the genotype that kept their stomata opened the longest. On day 17th of the drought stress, its $g_s/g_{s\ max}$ was remained higher than 50% while other genotypes had their $g_s/g_{s\ max}$ dropped lower than 50% since day 10th or 12th of the drought stress. However, it was the genotype B0057 still maintained $g_s/g_{s\ max}$ higher than 50% up to day 17th of the drought stress while the other genotypes contained their $g_s/g_{s\ max}$ lower than 50% since day 6th of the drought stress. Considering the progression of Ψ_{md} and stomatal regulation, the studied genotypes close their stomata at a rather high water potential. The $g_{s\ 12}$ in the year 2012 experiment occurred at the water potential of −0.13 ± 0.29 MPa while it occurred at much lower water potential in the year 2013 experiment (−1.41 ± 0.06 MPa). High $g_{s\ 12}$ found in 2012 was the result of a fast decreasing in $g_s/g_{s\ max}$ despite of a small change in Ψ_{md}. It is important to note that Ψ_{md} was maintained at a fairly high level for at least 10 days before it reached the tension of P_{12}. In addition, after the P_{12} was reached, more rapid evolutions in hydric traits were observed. This was consistent to a result of Lakso's study (1979) which shown a fast decline in stomata conductance at a critical Ψ which usually occurred between −1.8 to −2.2 MPa.

The stomata sensitivity to the drought stress ($g_{s\ 50}$) of the five genotypes from the experiment in 2012 was in agreement with the previous finding from Lauri *et al*. (2011); B0023, B0070 and B0111 were categorized as water spending genotypes while B0057 and B0097 were water saving genotypes. However, waned differences were found in stomatal regulation between the five genotypes in the 2013 experiment (Figure 40). The differences observed between two experimental years could be a result of the tree conditions. In 2012 experiment, the trees were only four months old before drought experiment began, the root system might not completely develop and there were few carbon reserves in the trees. This was not the case for the experiment in 2013 in which roots of these trees were more developed and there were some carbon reserves. Well-developed roots in the pots allowed more contacting surfaces for the trees to soil particles and more access to soil water. This could explain the higher Ψ_{md} and lower stem embolism rate that were generally found in the 2013 experiment when compared to the 2012 experiment.

Table 13. Xylem vulnerability to cavitation of the five apple genotypes from two different studies.

Genotype		B0070	B0057	B0111	B0097	B0023
P_{50} (MPa)	Lauri *et al.*, 2011	−6.77	−5.84	−3.70	−3.66	−2.52
	This study	−3.58	−3.25	−3.05	−3.08	−3.14

The pressure causing 50% loss of xylem conductivity (P_{50}) reported by Lauri *et al.* (2011) when compared to the P_{50} obtained in this study. The genotypes were presented according to their vulnerability to cavitation reported by Lauri *et al.* (2011) from the most tolerant to the most sensitive. In both studies, the values obtained used the same technique (Cavitron).

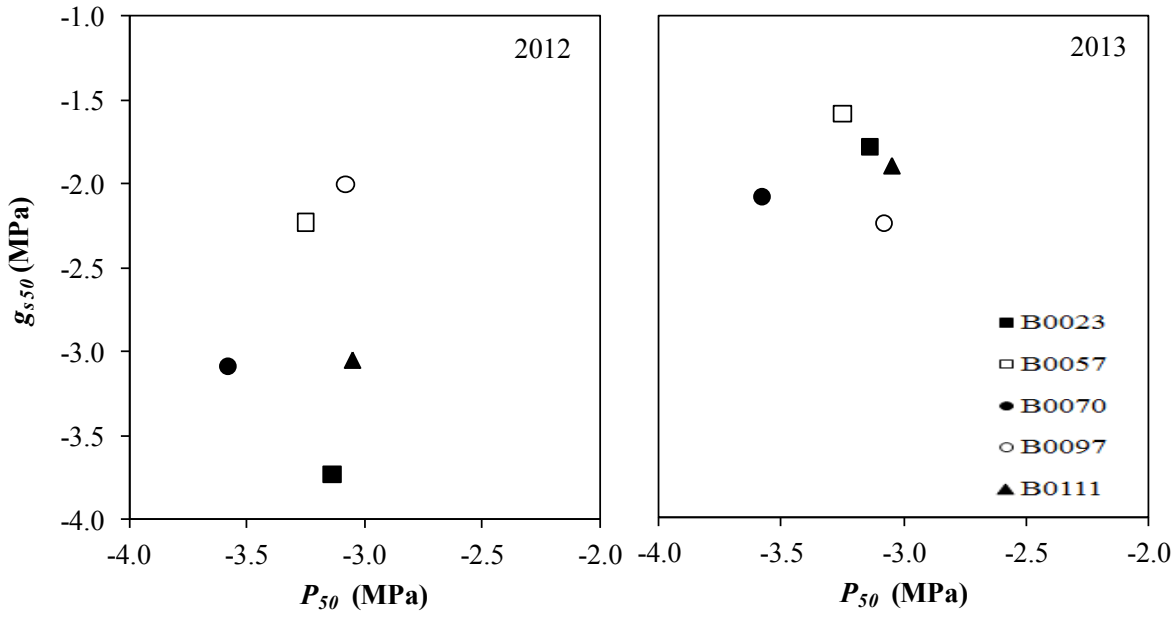

Figure 40. Variation in xylem vulnerability (P_{50}) and stomatal regulation ($g_{s\,50}$) of five apple genotypes.

The P_{50} and $g_{s\,50}$ of the five genotypes studied: B0023, B0057, B0070, B0097 and B0111 were presented. The P_{50}, a tension causing 50% loss of xylem conductivity, obtained from Cavitron while $g_{s\,50}$, a pressure causing 50% reduction of relative stomatal conductance, obtained from drought experiment in 2012 and 2013. There was no correlation found between the two variables (R^2 = 0.018 and 0.007 for 2012 and 2013 respectively).

The low and negative safety margin found in this study indicated that the apple trees chose to open their stomata despite the facing stress water deficit. This resulted consistently to a study of Beikircher *et al.* (2013) which suggested that apple cultivars tended to optimize the carbon gain by keeping their stomata opened. Since this study was carried out using the genotypes from a progeny which has not been submitted to any selection pressure, it was possible to find traits that might be rare to find from selected population. We also could not rule out a possibility of a methodological bias when calculating this safety margin because the pressure used for the calculation of $g_{s\ 88}$ was Ψ_{md}, measured on leaf whereas for P_{12} and P_{50}, the tension was in stem. Therefore, the negative safety margin had to be confirmed. It might be necessary to consider the traits measured on the same organ to calculate a safety margin.

The result of the study indicated that the drought stress affected *RGR* of B0057, B0097 and B0111. In case of B0111, the reduction *RGR* might be from the vulnerable conduits. The conduits of B0111 might experience greater tension when facing water deficit. Its water spending behavior was also vulnerable to cavitation, xylem dysfunction was evidence for this genotype. This could largely restrict growth when the tree exposed to the drought stress. Conversely, a significant reduction in *RGR* of B0057 and B0097 appeared to be a result of their water saving behavior. Although water saving strategy helped delaying cavitation and embolism, it also restricted gas exchange and might greatly limit the growth of tree. The result suggested a trade-off between growth and water saving behavior. However, this result could not determine the effect of drought stress on yield or tree survival. Therefore, further investigation also needed to assess these issues.

In conclusion, our results confirmed different combination strategies of drought tolerance which were previously reported for this progeny. Despite much narrower variation in cavitation resistance, the five genotypes still exhibited clear variability of stomata regulation in response to the drought stress. Since a trade-off with growth was only found on water saving behavior and not on vulnerability to cavitation of the apple trees, it suggested a selection for genotypes with the combination of water spending and cavitation resistant for the cultivation. The further investigation would be to identify the sources of variability of xylem vulnerability to cavitation and the effect of rootstock on this trait.

IV. General discussion

In this section, discussion on the within-species variation of xylem vulnerability to cavitation in three agronomic trees was raised and then the potential of using this trait as a criterion for drought tolerance screening would follow.

1. The variability of the xylem vulnerability to cavitation

The investigation of genetic variability of vulnerability to cavitation in agronomic species was the first objective of this PhD work. This was why this specific section devoted to the issue.

This research chose to study the three agronomic tree species: walnut tree, rubber tree and apple tree. These species are prominent in economical value, vastly cultivated all over the world, and drought threatening. The chosen species and their respective populations differed in various ways which offered more opportunities to explore the intraspecific variation in xylem vulnerability to cavitation. Walnut and apple trees originated from dryer regions of central Asia whereas the rubber tree was from tropical region of Brazil. These trees thus differed in drought sensitivity as seen from the distribution of the species. Walnut and apple trees could be cultivated in Mediterranean, semi-arid and arid areas while the cultivation areas of rubber tree are mostly limited within wetter environments of tropical region. The selected accessions (clone, cultivar or progenies from a cross) have been previously reported differently in various traits. In *Juglans* accessions, the variations were reported in water use efficiency (Aletà *et al*., 2009), budburst date, heat requirement and growth rate (Charrier *et al*., 2011; Poirier *et al*., 2004). In addition, the comparison between Persian (*J. regia*) and hybrid (*J. regia* x *J. nigra* NG 38) walnuts enhanced the genetic diversity of our studied populations and thus increased the chance to find variation of vulnerability to cavitation. This highlighted assumption arose from a great variation in vulnerability to cavitation when comparing *Prunus* species (Cochard *et al.*, 2008). The study found that P_{50} of the 10 *Prunus* species studied was ranked from −3.5 to −6.3 MPa. For the chosen ten rubber clones, despite that they all derived from small population, many studies shown high genetic polymorphism in the populations (Besse *et al*., 1994; Lekawipat *et al*., 2003), with differences in growth (Chandrashekar *et al*., 1998) and latex yielding (Priyadarshan *et al*., 2005). The two latter traits also found to vary in rubber clones grown in sub-optimal areas, indicatedputative differences between clones in drought tolerance. For the five apple genotypes, they were selected from 122 individuals of a progeny for their

contrasted vulnerability to cavitation and stomata response to drought which previously reported in Lauri *et al.*, (2011). Although the vulnerability to cavitation could vary according to the growth conditions, it expected that the selected genotypes to maintain the genetic differences at the least for cavitation resistance.

There was no significant difference between the populations of Persian and hybrid walnuts, the average P_{50} values were −1.97 and −2.16 MPa respectively. There were weak but significant differences in P_{50} when comparing the two species. Considering the variations of origin, parentage, phenology and water use efficiency of the walnut accessions studied, very similar vulnerability to cavitation among them is rather unexpected. This lack of variability in xylem vulnerability to cavitation within studied Persian walnut cultivars could explain by small different in their wood anatomical traits as these two traits are strongly linked (Ackerly, 2004; Hacke *et al*., 2000; Hacke *et al*., 2001a; Jacobsen *et al*., 2005). More vulnerability found in Persian walnuts compared with the hybrid walnuts could explain by the relationship between xylem vulnerability to cavitation and wood properties (Baas *et al*., 2004; Hacke *et al*., 2001; Markesteijn *et al*., 2011). Because high wood density related to higher reinforcement of xylem structure ($(t/b)^2$) which prevented the xylem implosion from high negative tension in xylem (Awad *et al*., 2010; Hacke *et al*., 2001a; Jacobsen *et al*., 2005), species with higher wood density assumed to be less vulnerable to cavitation. Various studies such as Chave *et al*., 2009; Poorter *et al*., 2010; Preston *et al*., 2006 suggest the invest correlationship between wood density and tree growth rate, thus the slow-growing hybrid walnut trees (Charrier *et al.*, 2011) were expected to be less vulnerable to cavitation when compared with Persian walnuts.

The ten rubber clones also exhibited similar xylem vulnerability to cavitation when compared their branch P_{50}; the values range was from −1.73 to −2.02 MPa. These P_{50} values were in the same range with other studies (Chen *et al*., 2010; Sangsing and Rattanawong, 2012; Sangsing *et al*., 2004) in which P_{50} varied from −1.22 to −2.42 MPa. These differences in P_{50} from the literatures might be a result of the differences in plant material used between the studies and the phenotypic plasticity of this trait. Studies of Sangsing and Rattanawong (2012) and Sangsing *et al.* (2004) were conducted on potted 1.5-year-old trees of two rubber clones: RRIM 600 and RRIT 251 and grown at Bangkok, Thailand. The P_{50} values found in these studies were higher (−1.22 to −1.91 MPa). In the study of Chen *et al.* (2010), which conducted in southern Yunnan of China, used two-year-old rubber tree from a local

commercial nursery. The study did not mention the name of clone used and trees were potted grown. Although there were significant differences found in P_{50} of the five apple genotypes studied, the diversity of this trait was much smaller when compared to the previous study by Lauri *et al.* (2011). It was rather disappointed that this progeny was chosen for their extent of the genetic variability, particularly on their contrast on vulnerability to cavitation. In the study of Lauri *et al.* (2011), the coefficient of variance of P_{50} was 0.39 while in this study, it was reduced to 0.07.

There was an assumption, on one hand, that the similarity or the small variations of xylem vulnerability to cavitation found on branches of walnut and rubber trees were a result of genetic canalization, at least within the commercial populations. The uniform selection and canalization in vulnerability to cavitation were first demonstrated on *Pinus* populations (Lamy *et al*., 2011; Lamy *et al*., 2014; Sáenz-Romero *et al*., 2013). There was no genetic variability in cavitation resistance found; neither between 513 genotypes of *Pinus pinaster* in a common garden (Lamy *et al.*, 2011) and along the environmental gradients from warm and dry to cooler and wetter sites nor between the populations of *Pinus hartwegii* along an altitudinal gradient, from 3,150 m to 3,650 m (Sáenz-Romero *et al.*, 2013). The average P_{50} was −3.73 and−3.42 MPa for the two studies respectively. This assumption is also applied to *Fagus sylvatica* (Wortemann *et al.*, 2011) which the cavitation resistance of 17 *Fagus sylvatica* populations which grew in provenance tests revealed a remarkable constant cavitation resistance across populations. The evidences from these studies suggested that genetic architecture could narrow trait variability to preserve functional phenotypes *in natura*. The agronomic species have generally undergone different selection pressure (Gepts, 2004; Purugganan and Fuller, 2009) and therefore the genetic variation in xylem vulnerability to cavitation could entirely differ from natural populations. Considering that resistance to cavitation usually came with trade-off such as high carbon cost for xylem structure and high hydraulic resistance, this trait might only contributed to the species survival and not entirely correlate to more desire traits of species with agronomic proposes. This might explain rather vulnerable and low variability of cavitation resistance in cultivated walnut accessions and rubber clones, while the apple genotypes which did not undergo the selection, showed higher variability for this trait.

On the other hand, there was assumption that narrow diversity of vulnerability to cavitation found between the apple genotypes was an effect of rootstock. Several studies demonstrated

that rootstock could modify hydric properties of the scion and result in different responses to drought stress (Atkinson *et al*., 2003; Bauerle *et al*., 2011; Feng *et al*., 2011; Jones *et al*., 1989; Tramontini *et al*., 2013), particularly on the modification of vessel diameter (Bauerle *et al*.,2011). The latter study found more vulnerable to cavitation from the scion grafted on dwarfing rootstock (including the M9 which was used in this study) when compared with grafted on vigorous rootstock. The study suggested that the differences in vulnerability to cavitation originated from different abilities to reduce vessel diameter in response to water deficit. A reduction in scion vessel diameter was observed from scion grafted on vigorous rootstock whereas it did not shift when using dwarfing rootstocks. This led to a consideration that grafting on dwarfing rootstock might mask the original adjustment of scion to cope with water stress and resulted in similar response among the genotypes. In addition, since there was no adjustment in response to drought stress, it resulted in weaker cavitation resistance. The suspicion on the influence of rootstock to vulnerability to cavitation of the scion found on apple trees brought forth the question on xylem vulnerability to cavitation of walnut and rubber trees. In commercial production, these two species ware also grafted on rootstocks: walnut cultivars are mostly grafted on seed-grown *J. regia*, occasionally *J. nigra* or *J. hindsii* while seed-grown RRIM 600 is mostly used as a rootstock in rubber production in Thailand. To date, the knowledge on responses of rootstock to drought stress, especially on the cavitation resistance is still lacking.

While most of the investigations on xylem vulnerability to cavitation were conducted on critical organs such as branches and stems, fewer were performed on petiole. In this study, the vulnerability to cavitation on petiole was conducted on rubber trees and clonal variation was found (P_{50} = −1.15 to −1.65 MPa). This finding led to a hypothesis proposal that the genetic canalization for this trait only held for critical organ bearing buds (branches or stems); but it would vary in less vital organ such as petiole. The finding of more vulnerable xylem vessels in petiole when compared with in branch indicating vulnerability segmentation also could be found on other species such as in *Populus balsamifera* and *Alnus glutinosa* (Hacke and Sauter, 1996), and in *Acer saccharinum* (Tsuda and Tyree, 1997). More vulnerable xylem in petiole would allow the vessels embolizing at a fairly high water potential and lead to leaf shedding. This ability let plant discarding transpiration organs in order to preserve more important ones from dehydration (Barigah *et al*., 2013; Zimmermann, 1983). The vulnerability segmentation also appears to hold true for walnut tree. Previous studies of Tyree *et al.* (1993) shown that petiole of *J. regia* cv Lara was more vulnerable to cavitation than its

branch (P_{50} = −1.15 and −1.40 MPa for petiole and branch, respectively). This result suggested that walnut tree allowed xylems in petiole embolizing and leaves shedding when facing drought stress. Therefore, more vulnerable conduits of petiole regarded as a benefit for the trees survival rather than a drawback.

2. The potential of using xylem vulnerability to cavitation in breeding programs

Pita *et al.* (2005) recommended the potential traits for drought tolerance screening were that; they should be easily assessed on the large-scale, allowed the identification of genotypic variation, had a sufficient heritability and allowed breeders identifying the best and the worst genotypes. In this study there were two aspects to consider whether xylem vulnerability to cavitation is a pertinent trait related to drought stress and it could be used for drought tolerance screening in breeding program: the technical and the scientific issues.

2.1 *The technical issue*

It was a benefit of current methods of Cavitron and air-injection that the time required for measuring the xylem vulnerability to cavitation has been shortened. The main advantage of these methods compared with the classical bench-drying method was that plant materials had not been required to expose to drought stress before they were used for the measurement. Instead, these current methods generated cavitation on non-stressed plant materials using centrifugal force and air pressurization. In addition, since it was possible to control a precise level of stress by adjusting the spinning velocity or the air pressure for the respective methods, it allowed us to complete a vulnerability curve using a single sample. Therefore, complete vulnerability curves could obtain from several samples within a day.

In this study, sigmoid shape vulnerability curves have been obtained when using samples with intact vessels at the center. Open-vessel artifact has been confirmed (Cochard *et al*., 2010; Martin-StPaul *et al*., 2014) by the experiments on walnut and rubber trees in which r-shape vulnerability curves were obtained on short sample segments. This problem restricted the use of Cavitron with long-vessels species such as ring porous species or most of tropical species that have long vessels. Fortunately, the sample size is not a limitation when using air-injection technique hence it can be applied on the species with long vessels. The air-injection technique considerably required more analytical time when compared with the Cavitron. At present,

only a single sample could be analyzed at a time with a set of pressure sleeve and perfusion tube. It considerably took more time to prepare and connect the sample with the system; when combining with time to induce embolism, only approximately five samples a day could be managed. However, as the equipments and the set up for this technique were far less sophisticated when compared with the Cavitron; it possible managed several samples at a time. This could be done either by multiplying the number of sleeves and perfusion tubes or by fabricating new sleeve that connected multiple samples. The idea is taken from the outlets of Xyl'EM apparatus which up to six samples could be attached and cavitation caouldbe induced on these samples at the same time. In addition, because the equipments used in air-injection technique couldbe easily moved and set for the measurement, it can be brought into a field and the measurement could be conducted shortly after samples were harvested. This might greatly reduced the artificial embolism from the preparation and transportation of the samples.

The effects of sample's age, light condition and sampling region observed in this study and in other studies (Cochard *et al*., 1999; Cochard *et al*., 2007; Herbette *et al*., 2010) highlighted the importance of the sampling design. To obtain as much as possible homogenous samples for the analysis, several precautions have to be seriously considered. It was also essential for the analysis conducted on samples with rather low and homogenous native embolism. This issue raised by Awad *et al.* (2010) suggested that if vulnerable curve constructed from stressed sample, the measurement would be done on the remaining functional vessels which were likely more resistant to cavitation. Therefore, the measurement underestimated the xylem vulnerability to cavitation of the sample. Moreover, it was also important to consider the directions of sap flow through sample when measuring vulnerability to cavitation with Cavitron. Significantly shifted was found in vulnerability to cavitation between the measurement with sense flow and antisense flow directions. This variation could be important to determine the variability of xylem vulnerability to cavitation of the species studied particularly if the difference for this trait was small between the studied accessions.

2.2 *The scientific issue*

In this study, despite the differences previously found on other traits and the genetic variation was expected to be large between studied populations, only weak intraspecific variation was found for xylem vulnerability to cavitation among the studied populations on their vital organs; branches for walnut and rubber trees, and stems for apple trees. It was still unclear

whether the selection for yield could affect vulnerability to cavitation of the species. In some studies, trade-off was found between cavitation resistance and productivity (Cochard *et al.*, 2007; Wikberg and Ogren, 2004) while in some other studies, no trade-off was found (Cochard, 2002; Sangsing *et al.*, 2004a; 2004b). In this study, we chose to experiment on young rubber and apple trees because drought stress could cause a severe damage or mortality especially in the orchard where irrigation limited. For this reason, we could only monitor the effect of drought stress on growth and not on yield. The effect of drought stress on rubber trees was not obvious between the stressed and the control trees for all clones. Reason for the similarity in *RGR* was presumably a result of plant phenology in which tree growth was normally halted during a dry period (Chandrashekar *et al*., 1998) and therefore the variation of *RGR* between control and stressed trees was not significant. On apple trees, small variation in P_{50} was responsible to no clear different in *RGR* between cavitation tolerance and sensitive genotypes.

Conversely, genetic variation of xylem vulnerability to cavitation found on petioles between ten rubber clones suggested that it could be used for the screening for drought tolerance. Vulnerable petioles allowed leaves to shed and prevented hydraulic failure in vital organs; this ability could be regarded as a desiccation avoidance trait of the species. Apart from genetic variation in vulnerability to cavitation of petiole, other traits related to desiccation avoidance including stomatal regulation and leaf shedding on rubber and apple trees were also observed. If cavitation resistance in critical organs was rather similar to the studied species, the future selection program can be firstly focused on these differences in drought avoidance traits. The selection for genotypes with high ability to avoid drought stress such as sensitive stomata and vulnerable xylems in petiole might help to prolong the occurrence of embolism in critical organs when faced drought episode.

It seemed to be a logical choice for the production in marginal areas to choose the genotypes that their vital organs rather vulnerable to cavitation, particularly on rubber trees. However, because these avoidance traits could limit gas exchange, adopting these behaviors might significantly halt tree growth during drought period as demonstrated by significant reduction of *RGR* of apple trees. The *RGR* of apple genotypes with water saving strategy significantly reduced during drought stress period while the effect on genotypes with water spending ability was not significant. This problem might not be a significant hiatus for fruit trees but in natural rubber production, growth rate especially during immature period was very important.

According to RRIT (2012), tapping (latex harvesting) can be done when rubber tree girth (stem diameter at 1.5 m above ground) is at least 0.50 m. Tapping on the immature trees could reduce latex production and tree growth. Therefore discontinuously growth during drought period could greatly prolong the immature period of rubber tree and affect the rubber production.

The averaged dry rubber yield of rubber clones in Thailand (RRIT, 2012) appeared positively linked with cavitation resistance of petiole found in this study. High-yielding clones producing dry rubber from 1,969 – 2,200 kg/ha/year such as RRIT 251, RRIT 408 and RRII 188 had more cavitation resistant petioles whereas lower yielding clones such as RRIM 600 (dry rubber yield = 1,644 kg/ha/year) had less cavitation resistant petioles.

Indeed, drought tolerance required an optimal combination of desiccation tolerance and desiccation avoidance to ensure yield and survival. Therefore, within these studied species, the accessions with moderate adaptation to desiccation avoidance might be chosen as a compromising method for drought tolerant selection while carrying out further investigation explored the vulnerability to cavitation of the vital organs on larger populations to broaden the genetic diversity beyond the commercial populations.

CONCLUSIONS AND PERSPECTIVES

The conclusions of this study were that small to none variations in xylem vulnerability to cavitation were found on the three species studied. The narrow variability of this trait on branches of two walnut species and rubber clones studied was a result of genetic canalization from uniform selection while rather small differences found on stems of apple genotypes were caused by the effect of rootstock inhibiting scion adjustment to water deficit. The hypothesis of genetic canalization of xylem vulnerability to cavitation was only applied to the critical organs bearing buds; however, on less vital organ (petioles) xylem vulnerability to cavitation was genetically varied and vulnerability segmentation was found. These findings needed to be thoroughly investigated by testing the respective variability for branches and petioles in several species including both deciduous and evergreen species.

The investigation should also consider other traits related to desiccation avoidance such as stomatal regulation and leaf shedding since genetic variation was found for these traits among the studied populations and they seemed to be more correlated with traits of agronomic interest (growth and yield) than the cavitation resistance of the critical organs. These traits related to desiccation avoidance can be considered as a first step on drought tolerance screening for our studied species while the next step would be an exploration of the vulnerability to cavitation on larger populations to broaden the genetic diversity beyond the commercial population.

Further investigation had to carry out to clarify source of the variability of vulnerability to cavitation, especially the environmental plasticity and the effects of rootstock. Firstly, the environmental plasticity; it might be more relevant to the selection program to confirm the responses to drought stress on field grown rubber and apple trees since this would reflect the actual environmental conditions that trees had to grow for commercial production. Moreover, the experiment on mature trees would also allow the evaluation of drought stress effects on yield which had not been done on this study. Secondly, from the result of narrower vulnerability to cavitation on stems of the five apple genotypes studied, we proposed that rootstock has modified the vulnerability to cavitation of scion. However, since the knowledge on response of rootstock to drought stress was still lacking thus further investigation was needed. Testing this hypothesis needed to compare the response to water deficit of own-root genotypes with grafted genotypes and might be also the compatibility of scions and rootstocks.

Regarding to the measurement of xylem vulnerability to cavitation, This study proposed a protocol with precautions for homogenous sampling and measurement techniques for the Cavitron as followed:

(i) Samples should be collected from non-stress trees hence low native embolism could be expected. Before the measurement of vulnerability to cavitation, representative populations had to be taken for native embolism measurement. It was important to measure the vulnerability to cavitation on samples with low and rather homogenous native embolism to avoid a bias from measuring vulnerability to cavitation on the remaining more resistance vessels.

(ii) Light condition, sample age and tree age might affect xylem vulnerability to cavitation of the samples. Apart from making sure that the samples were as much as possible homogenous in the age of samples themselves and the tree age, Sampling from the fully exposed to sunlight area such as the southern-side of the canopy or at the top of the canopy were recommended.

(iii) Samples should be harvested with possible maximum length; this would allow them to be re-cut to obtain a desire length for the analysis with minimum artificial embolism from the sample preparation. It might be necessary to do a cutting under tap water to prevent air infiltrating the vessels when the samples were prepared.

(iv) After samples were harvested, they should be immediately defoliated, wrapped in moist paper and plastic bag to minimize transpiration. Wrapping samples with wax or paraffin could help reducing transpiration from the samples, particularly for a long period of transportation. The harvested samples should be kept in cold storage (approximately 4°C) while they were waiting for the analysis. It was previously proposed that the maximum storage time was up to 30 days after the harvest for the measurement of xylem vulnerability to cavitation; however, this value might be varying according to the species and storage conditions. It might necessary to verify the maximum storage time for the species studied or closely observed the variation of xylem vulnerability to cavitation measured between the measurement dates.

(v) The segments, which would be used for the measurement of vulnerability to cavitation, should contain only the intact vessels at their center. Using segments with open-to-center vessels could result in the overestimation of vulnerability to cavitation from open vessel artifact. This open vessel artifact could be checked by

infiltrating one end of the sample with compressed air while another end submerging under water. If vessels were opened to center, air bubbles would be observed from the submerged end. Only samples with intact vessels should be used for the analysis.

(vi) Directions of water flow through the segments while they were spinning on Cavitron could cause the variation of vulnerability to cavitation thus all the measurements should be conducted with similar flow direction. Here, working with the antisense flow where bigger water reservoir was placed at the distal end of segment and smaller reservoir at the proximal end was recommended. The reason for this recommendation was that because the sense flow could result in a slightly faster increasing in embolism rate due to a higher number of cut open vessels at the proximal end that quickly embolized when nuclei were seeded into them by measurement flows.

This protocol should ensure the accurate estimation of xylem vulnerability to cavitation using Cavitron technique.

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Variabilité génétique de la tolérance à la sécheresse d'arbres d'intérêts agronomiques: rôle de la vulnérabilité à la cavitation du xylème.

Résumé

Dans un contexte de changements climatiques, le stress hydrique et la gestion de l'eau sont considérées comme une contrainte importante pour le secteur agricole. Ainsi la sélection pour la tolérance à la sécheresse est devenue un objectif majeur pour de nombreux programmes de sélection. La vulnérabilité à la cavitation est considérée comme un trait d'intérêt pour la sélection à une sécheresse extrême, en particulier pour les plantes ligneuses. Pourtant, l'étendue de sa variabilité et sa relation avec la tolérance à la sécheresse sont mal documentées à l'échelle intra-spécifique, et en particulier dans les espèces cultivées. Dans cette étude, la variabilité génétique de la vulnérabilité à la cavitation a été étudiée sur trois dispositifs expérimentaux différents avec trois arbres d'intérêt agronomique qui sont menacés par le risque de sécheresse: noyer, hévéa et pommier. Une faible ou aucune, variation de la vulnérabilité à la cavitation a été trouvée dans les espèces étudiées ainsi qu'entre deux espèces de noyers malgré les différences de traits précédemment rapportés. Ces résultats suggèrent une canalisation de la résistance à la cavitation dans les organes critiques (branches, tiges). Chez le pommier, le porte-greffe est soupçonné de provoquer de la plus étroite résistance à la cavitation sur le greffon. Par contre, des différences ont bien étés trouvées sur d'autres traits de réponse à la sécheresse comme la régulation stomatique, la chute des feuilles ou encore la vulnérabilité à la cavitation dans le pétiole. Ainsi, la vulnérabilité à la cavitation des organes critiques n'est pas un paramètre pertinent pour la sélection de la tolérance à la sécheresse, et les programmes de sélection sur les espèces étudiées ne semblent pas avoir affecté ce paramètre.

Mots-clés: conductance stomatique, *Hevea brasiliensis*, hydraulique, *Juglans* spp, *Malus domestica*, stress hydrique, sécheresse, vulnérabilité à la cavitation.

Genetic variability of drought tolerance of trees of agronomic interest: the role of vulnerability to xylem cavitation.

Abstract

In a context of climatic changes, drought stress and water management are regarded as one of the most important constraints for agricultural sector. Thus the selection for drought tolerance became a main objective for many breeding programs. Vulnerability to cavitation is considered a trait of interest for the selection for extreme drought stress, especially for woody species. However, the extent of its variability and its relation to drought tolerance are poorly documented on intraspecific level, particularly for cultivated species. In this study the genetic variability of vulnerability to cavitation was studied on three different experimental devices with three trees of agronomic interest that are threatened by the risk of drought: walnut, rubber and apple trees. Low or no variation in xylem vulnerability to cavitation was found in the studied species and between two species of walnuts despite differences previously reported features. These results suggested a canalization of cavitation resistance on critical organs (branches and stems). In apple tree, the rootstock was suspected to cause the narrow resistance to cavitation on the scion. On the contrary, differences on other traits in response to drought stress such as stomatal regulation, leaf shedding or vulnerability to cavitation on petiole were found. Therefore, vulnerability to cavitation of critical organs was not a relevant parameter for the selection of drought tolerance and breeding programs on the species studied did not appear to affect this parameter.

Keywords: drought, *Hevea brasiliensis*, hydraulic, *Juglans* spp., *Malus domestica*, stomatal conductance, vulnerability to cavitation, water stress.